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(71) Applicants *(for all designated States except US)*: **ROCHE DIAGNOSTICS GMBH** [DE/DE]; Sandhofer Strasse 116, D-68305 Mannheim (DE). **INSTITUT PASTEUR** [FR/FR]; 25-28, rue du Docteur Roux, F-75724 Paris Cedex 15 (FR). **PHARMA-WALDHOF GMBH & CO.KG** [DE/DE]; Hansa-Allee 159, D-40549 Düsseldorf (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TISCHER, Wilhelm** [DE/DE]; Finkenweg 5, D-82380 Peißenberg (DE). **IHLENFELDT, Hans-Georg** [DE/DE]; Rabenkopfstrasse 5, D-82393 Iffeldorf (DE). **BARZU, Octavian** [FR/FR]; 3, allée de la Bougainvillée, F-92160 Antony (FR). **SAKAMOTO, Hiroshi** [JP/FR]; 3, sentier des Jardies, F-92190 Meudon (FR). **PISTOTNIK, Elisabeth** [FR/FR]; 11, rue de la Fosse aux Moines, F-94000 Créteil (FR). **MARLIERE, Philippe** [FR/FR]; 2, allée Saint Martin, F-91450 Etiolles (FR). **POCHET, Sylvie** [FR/FR]; 5, rue Gossec, F-75012 Paris (FR).

(74) Agents: **WEICKMANN, H.** et al.; Kopernikusstrasse 9; D-81679 München (DE).

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(54) Title: ENZYMATIC SYNTHESIS OF DEOXYRIBONUCLEOSIDES

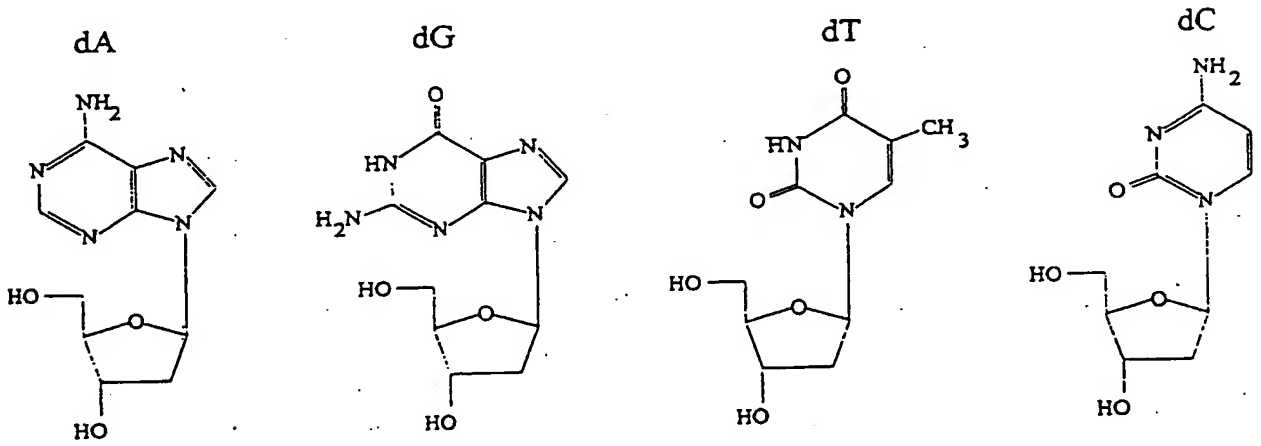
(57) Abstract: The present invention relates to a method for the in vitro enzymatic synthesis of deoxyribonucleosides and enzymes suitable for this method.

Enzymatic synthesis of deoxyribonucleosides

Description

The present invention relates to a method for the in vitro enzymatic synthesis of deoxyribonucleosides and enzymes suitable for this method.

Natural deoxyribonucleosides (deoxyadenosine, dA; deoxyguanosine, dG; deoxycytidine, dC and thymidine, dT) are building blocks of DNA. The N-glycosidic bond between nucleobase and sugar involves the N_1 of a pyrimidine or the N_9 of a purine ring and the C_1 of deoxyribose.



In the living cells the four deoxyribonucleosides (dN) result from the "salvage pathway" of nucleotide metabolism. A group of enzymes is involved in cellular catabolism of deoxyribonucleosides. Besides deoxyriboaldolase (EC 4.1.2.4) and deoxyribomutase (EC 2.7.5.1), this group also includes thymidine phosphorylase (EC 2.4.2.4) and purine nucleoside phosphorylase (EC 2.4.2.1). These four enzymes are induced by the addition of deoxyribonucleosides to the growth medium. The genes

coding for these enzymes have been shown to map closely together on the bacterial chromosome (Hammer-Jesperson and Munch-Peterson, Eur.J.Biochem.17 (1970), 397 and literature cited therein). In E.coli the genes as described above are located on the deo operon which exhibits an unusual and complicated pattern of regulation (Valentin-Hansen et al., EMBO J.1 (1982), 317).

Using the enzymes of the deo operon for synthesis of deoxynucleosides was described by C.F.Barbas III (Overproduction and Utilization of Enzymes in Synthetic Organic Chemistry, Ph.D. Thesis (1989), Texas A&M University). He applied phosphopentomutase and thymidine phosphorylase for the synthesis of deoxynucleosides. Deoxyribose 5-phosphate was prepared by chemical synthesis (Barbas III et al., J.Am.Chem.Soc. 112 (1990), 2013-2014), which makes this compound expensive as starting material and not suitable for large scale synthesis. He also made deoxyriboaldolase available as a recombinant enzyme and investigated its synthetic applicability but neither he nor C.-H.Wong (Microbial Aldolases in Carbohydrate Synthesis: ACS Symp.Ser.No.466: Enzymes in Carbohydrate Synthesis, Eds. M.D.Bednarski, E.S.Simon (1991), 23-27) were able to carry out a coupled one-pot synthesis employing all three enzymes. It appears likely that some drawbacks exist which could not be circumvented. Among these drawbacks are insufficient chemical equilibrium, instability of intermediates, such as deoxyribose 1-phosphate and inactivation and inhibition effects of involved compounds on the enzymes.

Evidence of an advantageous equilibrium is given by S.Roy et al. (JACS 108 (1986), 1675-78). For the aldolase reaction the equilibrium is on the desired product side (deoxyribose 5-phosphate), for the phosphopentomutase it is on the wrong side (also deoxyribose 5-phosphate) and for the purine nucleoside phosphorylase it is on the desired synthesis product side. The authors suggest coupling of the three enzyme reactions to obtain reasonable yields. Contrary to these suggestions they prepared deuterated

deoxyguanosine and thymidine in a two step procedure, that is deoxyribose 5-phosphate in a first step and deoxynucleoside in a second step. Isolated yields of the second step were 11% and 5% for deoxyguanosine and thymidine, respectively. These low yields are also obtained in the preparation of arabinose-based nucleosides (Barbas III (1990), supra). These low yields indicate serious drawbacks for the use of the enzymes of the deo operon in a synthetic route which have to work in the reverse direction of their biological function, which is degradation of deoxynucleosides.

Thus, there does not exist any economical commercial method at present for the enzymatic in vitro synthesis of deoxyribonucleosides. Hitherto, for commercial purposes, deoxynucleosides are generated from fish sperm by enzymatic cleavage of DNA. This method, however, involves several disadvantages, particularly regarding difficulties of obtaining the starting material in sufficient quantity and quality.

Therefore, it was an object of the invention to provide a method, by means of which the drawbacks of the prior are eliminated at least partially and which allows efficient and economical synthesis of deoxyribonucleosides without any dependence on unreliable natural sources.

Surprisingly, it was found that the drawbacks of previous enzymatic synthesis routes can be avoided and deoxyribonucleosides can be obtained in high yields of e.g. at least 80% based on the amount of starting material.

In a first aspect, the present invention relates to a method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising reacting deoxyribose 1-phosphate (dR1P) and a nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed.

The reaction is catalyzed by an enzyme which is capable of transferring a deoxyribose moiety to a nucleobase, with a deoxyribonucleoside being formed. Preferably, the reaction is catalyzed by a thymidine phosphorylase (TP, EC 2.4.2.4) or a purine nucleoside phosphorylase (PNP, EC 2.4.2.1). For the EC designation of these enzymes and other enzymes mentioned below reference is made to the standard volume Enzyme Nomenclature 1992, Ed. E.C.Webb, Academic Press, Inc.

These enzymes and other enzymes mentioned below are obtainable as native proteins from natural sources, i.e. any suitable organisms selected from eukaryotes, prokaryotes and archaea including thermophilic organisms. Further, these enzymes are obtainable as recombinant proteins from any suitable host cell which is transformed or transfected with a DNA encoding said enzyme. The host cell may be a eukaryotic cell, a prokaryotic cell or an archaea cell. Particular preferred sources of native or recombinant TP or PNP are prokaryotic organisms such as E.coli. Recombinant TP may be isolated from E.coli strain pHSP 282 (CNCM I-2186) deposited on April 23, 1999, which is a recombinant E.coli strain transformed with a plasmid containing the E.coli deoA (thymidine phosphorylase) insert. Recombinant PNP may be isolated from E.coli strain pHSP 283 (CNCM I-2187) deposited on April 23, 1999, which is a recombinant E.coli strain transformed with a plasmid containing the E.coli deoD (purine nucleoside phosphorylase) insert. The nucleotide sequence of the TP gene and the corresponding amino acid sequence are shown in SEQ ID NO.1 and 2. The nucleotide sequence of the PNP gene and the corresponding amino acid sequence are shown in SEQ ID NO.15 and 16 and 3 and 4.

The nucleobase, to which the deoxyribose unit is transferred, will be selected from any suitable nucleobase. For example, the nucleobase may be a naturally occurring nucleobase such as thymine, uracil, adenine, guanine or hypoxanthine. It should be noted, however, that also non-naturally occurring analogs thereof are suitable as enzyme substrates such

as 2-thio-uracil, 6-aza-uracil, 5-carboxy-2-thiouracil, 6-aza-thymine, 6-aza-2-thio-thymine and 2,6-diamino-purine.

Preferably the inorganic phosphate is removed from the reaction. This removal is preferably effected by (i) conversion to inorganic pyrophosphate, (ii) precipitation/complexation and/or (iii) substrate phosphorylation.

Conversion to inorganic pyrophosphate may be effected by a phosphate transfer from a phosphorylated, preferably polyphosphorylated substrate such as fructose diphosphate (FDP), wherein a phosphate group is cleaved from the phosphorylated substrate and reacts with the inorganic phosphate, with inorganic pyrophosphate (PPi) being formed. This phosphate transfer is preferably catalyzed by a PPi-dependent phosphorylase/kinase, e.g. by a PPi-dependent phosphofructokinase (PFK-PPi, EC 2.7.1.90), which catalyzes the reaction of fructose diphosphate (FDP) and inorganic phosphate to fructose 6-phosphate (F6P) and inorganic pyrophosphate. Preferred sources of PPi-dependent kinases/phosphorylases and genes coding therefor are from Propionibacterium freudenreichii (shermanii) or from potato tubers.

Further, the inorganic phosphate may be removed from the reaction by precipitation and/or complexation which may be effected by adding polyvalent metal ions, such as calcium or ferric ions capable of precipitating phosphate or by adding a complex-forming compound capable of complexing phosphate. It should be noted that also a combination of pyrophosphate formation and complexation/ precipitation may be carried out.

Furthermore, the removal of inorganic phosphate may be effected by substrate phosphorylation. Thereby the inorganic phosphate is transferred to a suitable substrate, with a phosphorylated substrate being formed. The substrate is preferably selected from saccharides, e.g. disaccharides such as sucrose or maltose. When using disaccharides as substrate, a

monosaccharide and a phosphorylated monosaccharide are obtained. The phosphate transfer is catalyzed by a suitable phosphorylase/kinase such as sucrose phosphorylase (EC 2.4.1.7) or maltose phosphorylase (EC 2.4.1.8). Preferred sources of these enzymes are Leuconostoc mesenteroides, Pseudomonas saccherophila (sucrose phosphorylase) and Lactobacillus brevis (maltose phosphorylase).

The phosphorylated substrate may be further reacted by additional coupled enzymatic reactions, e.g. into a galactoside (Ichikawa et al., Tetrahedron Lett.36 (1995), 8731-8732). Further, it should be noted that phosphate removal by substrate phosphorylation may also be coupled with other phosphate removal methods as described above.

Deoxyribose 1-phosphate (dR1P), the starting compound of the method of the invention, is a rather unstable compound, the isolation of which is difficult. In a preferred embodiment of the present invention, d1RP is generated in situ from deoxyribose 5-phosphate (dR5P) which is relatively stable at room temperature and neutral pH. This reaction is catalyzed by a suitable enzyme, e.g. a deoxyribomutase (EC 2.7.5.1) or a phosphopentose mutase (PPM, EC 5.4.2.7) which may be obtained from any suitable source as outlined above. The reaction is preferably carried out in the presence of divalent metal cations, e.g. Mn^{2+} or Co^{2+} as activators. Preferred sources of deoxyribomutase are enterobacteria. Particular preferred sources of native or recombinant PPM are prokaryotic organisms such as E.coli. Recombinant PPM may be isolated from E.coli strain pHSP 275 (CNCM I-2188) deposited on April 23, 1999, which is a recombinant E.coli strain transformed with a plasmid containing the E.coli deo B (phosphopentose mutase) insert. The nucleotide sequence of the PPM gene and the corresponding amino acid sequence are shown in SEQ ID NO.17 and 18 and 5 and 6.

dR5P may be generated by a condensation of glyceraldehyde 3-phosphate (GAP) with acetaldehyde. This reaction is catalyzed by a suitable enzyme, preferably by a phosphopentose aldolase (PPA, EC 4.1.2.4). The reaction exhibits an equilibrium constant favorable to the formation of the phosphorylated sugar (K_{eq} = [dR5P]/[acetaldehyde] x [GAP] = 4.2 x 10^3 x M^{-1}). PPA forms an unstable Schiff base intermediate by interacting with the aldehyde. Particular preferred sources of native or recombinant PPA are prokaryotic organisms such as E.coli. Recombinant PPA may be isolated from E.coli strain pHSP 276 (CNCM I-2189) deposited on April 23, 1999. This recombinant E.coli strain is transformed with a plasmid containing the deoC (phosphopentosealdolase) insert. The nucleotide sequence of the PPA gene and the corresponding amino acid sequence are shown in SEQ ID NO.19 and 20 and 7 and 8.

GAP is a highly unstable compound and, thus, should be generated in situ from suitable precursors which are preferably selected from fructose 1,6-diphosphate (FDP), dihydroxyacetone (DHA) and/or glycerolphosphate (GP), with FDP being preferred.

FDP can be converted by an FDP aldolase (EC 4.1.2.13) selected from FDP aldolases I and FDP aldolases II to GAP and dihydroxyacetone phosphate (K_{eq} = [FDP]/[GAP] x [DHAP] = $10^4 M^{-1}$). The two families of FDP aldolases giving identical end products (GAP and DHAP) via two chemically distinct pathways may be used for this reaction. FDP aldolase I forms Schiff base intermediates like PPA, and FDP aldolase II which uses metals (Zn^{2+}) covalently bound to the active sites to generate the end products. FDP-aldolase I is characteristic to eukaryotes, although it is found in various bacteria. FDP-aldolase II is more frequently encountered in prokaryotic organisms. If FDP-aldolase reacts with FDP in the presence of acetaldehyde, the latter compound can interact with DHAP to yield an undesired condensation by-product named deoxyxylolose 1-phosphate (dX1P). Thus,

the reaction is preferably conducted in a manner by which the generation of undesired side products is reduced or completely suppressed.

Particular preferred sources of native or recombinant FDP aldolases are prokaryotic or eukaryotic organisms. For example, FDP aldolase may be isolated from rabbit muscle. Further, FDP aldolase may be obtained from bacteria such as E.coli. Recombinant FDP aldolase may be isolated from recombinant E.coli strain pHSP 284 (CNCM I-2190) which is transformed with a plasmid containing the E.coli fba (fructose diphosphate aldolase) insert. The nucleotide sequence of the E.coli FDP aldolase gene and the corresponding amino acid sequence are shown in SEQ ID NO.9 and 10.

On the other hand, GAP may be generated from DHAP and ATP, with dihydroxyacetone phosphate (DHAP) and ADP being formed and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerokinase (GK, EC 2.7.1.30) and a triose phosphate isomerase (TIM, EC 5.3.1.1). Suitable glycerokinases are obtainable from E.coli, suitable triose phosphate isomerases are obtainable from bovine or porcine muscle.

In a still further embodiment of the present invention GAP may be generated from glycerol phosphate (GP) and O_2, with DHAP and H_2O_2 being formed and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerophosphate oxidase (GPO, EC 1.1.3.21) and a triose phosphate isomerase (TIM, EC 5.3.1.1). Suitable glycerophosphate oxidases are obtainable from Aerococcus viridans.

In an alternative embodiment of the present invention deoxyribose 5-phosphate (dR5P) is generated by phosphorylation of deoxyribose. Preferably this reaction is carried out in the presence of a suitable enzyme, e.g. a deoxyribokinase (dRK, EC 2.7.1.5) which may be obtained from prokaryotic organisms, particularly Salmonella typhi and in the presence of

ATP. The nucleotide sequence of the Salmonella dRK gene and the corresponding amino acid sequence are shown in SEQ ID NO.11 and 12.

By the reaction as outlined above deoxyribonucleosides are obtained which contain a nucleobase which is accepted by the enzymes TP and/or PNP. TP is specific for thymidine (T), uracil (U) and other related pyrimidine compounds. PNP uses adenine, guanine, hypoxanthine or other purine analogs as substrates.

The synthesis of deoxyribonucleosides which are not obtainable by direct condensation such as deoxycytosine (dC), thus, require an additional enzymatic reaction, wherein a deoxyribonucleoside containing a first nucleobase is reacted with a second nucleobase, with a second ribonucleoside containing the second nucleobase being formed. The second nucleobase is preferably selected from cytosine and analogs thereof such as 5-azacytosine. It should be noted, however, that also other nucleobases such as 6-methyl purine, 2-amino-6-methylmercaptopurine, 6-dimethylaminopurine, 2,6-dichloropurine, 6-chloroguanine, 6-chloropurine, 6-azathymine, 5-fluorouracil, ethyl-4-amino-5-imidazole carboxylate, imidazole-4-carboxamide and 1,2,4-triazole-3-carboxamide may be converted to the corresponding deoxyribonucleoside by this nucleobase exchange reaction (Beaussire and Pochet, Nucleosides & Nucleotides 14 (1995), 805-808, Pochet et al., Bioorg.Med.Chem.Lett.5 (1995), 1679-1684, Pochet and Dugué, Nucleosides & Nucleotides 17 (1998), 2003-2009, Pistotnik et al., Anal.Biochem.271 (1999), 192-199). This reaction is preferably catalyzed by an enzyme called nucleoside 2-deoxyribosyltransferase (NdT, EC 2.4.2.6) which transfers the glycosyl moiety from a first deoxynucleoside to a second nucleobase, e.g. cytosine. A preferred source of native or recombinant NdT are prokaryotic organisms such as lactobacilli, particularly Lactobacillus leichmannii. Recombinant NdT may be isolated from recombinant E.coli strain pHSP 292 (CNCM I-2191) deposited on April 23, 1999, which is transformed with a plasmid

containing the L.leichmannii NdT (nucleoside 2-deoxyribosyltransferase) insert. The nucleotide sequence of the NdT gene and the corresponding amino acid sequence are shown in SEQ ID NO.13 and 14.

A further aspect of the present invention is a method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of: (i) condensing glyceraldehyde 3-phosphate (GAP) with acetaldehyde to deoxyribose 5-phosphate (dR5P), (ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate (dR1P) and (iii) reacting deoxyribose 1-phosphate and a nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed. Preferably, the reaction is carried out without isolating intermediate products and, more preferably, as a one-pot reaction. Further, the removal of the inorganic phosphate from the reaction is preferred.

As outlined above, the glyceraldehyde 3-phosphate may be generated from FDP, DHA and/or GP. Preferably, FDP is used as a starting material.

In order to avoid the production of undesired by-products and the toxic effects of acetaldehyde, the course of the reaction is preferably controlled by suitable means. Thus, preferably, the reaction is carried out in a manner such that the acetaldehyde concentration in step (ii) is comparatively low, e.g. less than 100 mM, particularly less than 50 mM, e.g. by adding the acetaldehyde in portions or continuously during the course of the reaction and/or by removing excess acetaldehyde. Further, it is preferred that before step (ii) excess starting materials and/or by-products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate (dX1P), are removed. This removal may be effected by chemical and/or enzymatic methods, e.g. precipitating FDP with ferric salts or enzymatically degrading X1P via dihydroxyacetone phosphate. Alternatively or additionally the reaction conditions may be adjusted such that before step (ii) no substantial amounts, preferably less than 10 mM, of starting materials and/or by-

products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate, are present in the reaction mixture.

In still another embodiment, the present invention relates to a method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of: (i) phosphorylating deoxyribose to deoxyribose 5-phosphate, (ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate and (iii) reacting deoxyribose 1-phosphate and nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed. Preferably, these reactions are carried out with isolating intermediate products and, more preferably, as a one-pot reaction. To obtain a better yield the removal of inorganic phosphate from step (iii) is preferred.

By the process as described above naturally occurring deoxyribonucleosides such as dA, dG, dT, dU and dT but also analogs thereof containing non-naturally occurring nucleobases and/or non-naturally occurring deoxyribose sugars such as 2'-deoxy-3'-azido-deoxyribose or 2'-deoxy-4'-thio-deoxy-ribose may be produced.

The deoxyribonucleosides obtained may be converted to further products according to known methods. These further reaction steps may comprise the synthesis of deoxyribonucleoside mono-, di- or triphosphates, of H-phosphonates or phosphoramidites. Additionally or alternatively, labelling groups such as radioactive or chemical labelling groups may be introduced into the deoxyribonucleosides.

Still a further aspect of the present invention is the use of an isolated nucleic acid molecule encoding a nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6) for the preparation of an enzyme in an in vitro enzymatic synthesis process, wherein a deoxyribonucleoside containing a first nucleobase is reacted with a second nucleobase, with a deoxyribonucleoside containing the second nucleobase being formed. The

second nucleobase is preferably selected from cytidine and analogs thereof, 2,6-dichloro-purine, 6-chloro-guanine, 6-chloro-purine, 6-aza-thymine and 5-fluoro-uracil. The first nucleobase is preferably selected from thymine, guanine, adenine or uracil.

More preferably, the nucleic acid molecule encoding an NdT comprises (a) the nucleotide sequence shown in SEQ ID NO.13 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of degeneracy of the genetic code or (c) the nucleotide sequence hybridizing under stringent conditions to the sequence (a) and/or (b). Apart from the sequence of SEQ ID NO.13 the present invention also covers nucleotide sequences coding for the same polypeptide, i.e. they correspond to the sequence within the scope of degeneracy of the genetic code, and nucleotide sequence hybridizing with one of the above-mentioned sequences under stringent conditions. These nucleotide sequences are obtainable from SEQ ID NO.13 by recombinant DNA and mutagenesis techniques or from natural sources, e.g. from other Lactobacillus strains.

Stringent hybridization conditions in the sense of the present invention are defined as those described by Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), 1.101-1.104. According to this, hybridization under stringent conditions means that a positive hybridization signal is still observed after washing for one hour with 1 x SSC buffer and 0.1% SDS at 55°C, preferably at 62°C and most preferred at 68°C, in particular, for one hour in 0.2 x SSC buffer and 0.1% SDS at 55°C, preferably at 62°C and most preferred at 68°C.

Moreover, the present invention also covers nucleotide sequences which, on nucleotide level, have an identity of at least 70%, particularly preferred at least 80% and most preferred at least 90% to the nucleotide sequence shown in SEQ ID NO.13. Percent identity are determined according to the following equation:

$$I = \frac{n}{L} \times 100$$

wherein I are percent identity, L is the length of the basic sequence and n is the number of nucleotide or amino acid difference of a sequence to the basic sequence.

Still another subject matter of the present invention is a recombinant vector comprising at least one copy of the nucleic acid molecule as defined above, operatively linked with an expression control sequence. The vector may be any prokaryotic or eukaryotic vector. Examples of prokaryotic vectors are chromosomal vectors such as bacteriophages (e.g. bacteriophage Lambda) and extrachromosomal vectors such as plasmids (see, for example, Sambrook et al., supra, Chapter 1-4). The vector may also be a eukaryotic vector, e.g. a yeast vector or a vector suitable for higher cells, e.g. a plasmaid vector, viral vector or plant vector. Suitable eukaryotic vectors are described, for example, by Sambrook et al., supra, Chapter 16. The invention moreover relates to a recombinant cell transformed with the nucleic acid or the recombinant vector as described above. The cell may be any cell, e.g. a prokaryotic or eukaryotic cell. Prokaryotic cells, in particular, E.coli cells, are especially preferred.

The invention refers to an isolated polypeptide having NdT activity encoded by the above-described nucleic acid and its use for the preparation of deoxyribonucleosides. Preferably, the polypeptide has the amino acid sequence shown in SEQ ID NO.14 or an amino acid sequence which is at least 70%, particulary preferred at least 80% and most preferred at least 90% identical thereto, wherein the identity may be determined as described above.

Finally, the present invention also relates to the use of isolated nuclecic acid molecules having thymidine phosphorylase (TP), purine nucleoside phosphorylase (PNP), phosphopentose mutase (PPM), phosphopentose aldolase (PPA), FDP aldolase and deoxyribokinase (dRK) activity for the preparation of an enzyme for a method for the in vitro synthesis of deoxynucleosides. Preferably, these nucleic acids are selected (a) from a nucleotide sequence shown in SEQ ID NO.1, 3, 5, 7, 9 or 11 or their complementary sequences, (b) a nucleotide sequence corresponding to a sequence of (a) within the scope of degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to a sequence (a) and/or (b).

Isolated polypeptides having TP, PNP, PPM, PPA, FDP aldolase or dRK activity encoded by the above-described nucleic acids may be used for the preparation of deoxyribonucleosides. Preferably, these polypeptides have the amino acid sequence shown in SEQ ID NO.2, 4, 16, 6, 18, 8, 20, 10 or 12 or an amino acid sequence which is at least 70%, particulary preferred at least 80% and most preferred at least 90% identical thereto, wherein the identity may be determined as described above.

An isolated nucleic acid molecule encoding a dRK may be used for the preparation of an enzyme for an in vitro method for the enzymatic synthesis of deoxyribonucleosides comprising the step of phosphorylating deoxyribose to deoxyribose 5-phosphate, wherein said nucleic acid molecule comprises (a) the nucleotide sequence shown in SEQ ID NO.11 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b). Correspondingly, an isolated polypeptide having dRK activity is suitable for an in vitro method for the enzymatic synthesis of deoxyribonucleosides as outlined above.

The E.coli strains pHSP 282 (CNCM I-2186), pHSP 283 (CNCM I-2187), pHSP 275 (CNCM I-2188), pHSP 276 (CNCM 2189), pHSP 284 (CNCM I-2190) and pHSP 292 (CNCM I-2191) were deposited according to the regulations of the Budapest Treaty on April 23, 1999 at the Collection Nationale de Culture de Microorganismes, Institut Pasteur, 25, Rue de Docteur Roux, 75724 Paris Cedex 15.

Description of figures

Figure 1 shows the synthesis of dR5P according to Example 12.

Figure 2 shows the synthesis of deoxyadenosine according to Example 12.

Figure 3 shows the synthesis of deoxyadensine according to Example 13.

Figure 4 shows the synthesis of dG-NH_2 according to Example 14.

Example 1

Sources of Enzymes

L-glycerol 3-phosphate oxidase (1.1.3.21) from Aerococcus viridans, sucrose phosphorylase (2.4.1.7), fructose 6-phosphate kinase (2.7.1.90) from Propionibacterium freudenreichii, rabbit muscle aldolase (RAMA), formate dehydrogenase, glycerolphosphate dehydrogenase (GDH), triosephosphate isomerase (TIM), catalase, glycerol 3-phosphate oxidase and maltose phosphorylase were obtained from commercial sources (Roche Diagnostics, Sigma) or as described in the literature.

FDP aldolase II (4.1.2.13), phosphopentose aldolase (PPA, EC 4.1.2.4), phosphopentose mutase (PPM, EC 5.4.2.7), thymidine phosphorylase (TP,

EC 2.4.2.4), purine nucleoside phosphorylase (PNP, EC 2.4.2.1), nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6) were obtained from E.coli strains deposited at CNCM (see above).

Example 2

Protocol of the synthesis of deoxyadenosine

Reaction mixture A was prepared by adding acetaldehyde (final concentration 250 mM), FDP aldolase II (0.5 U/ml), PPA (2.5 U/ml) to 20 ml of 100 mM fructose-1,6-diphosphate (FDP), pH 7.6 and incubating overnight at 4°C.

Mixture B was prepared by adding $MnCl_2$ (final concentration 0.6 mM), glucose 1,6-diphosphate (15 μM), PPM (1.5 U/ml), PNP (0.4 U/ml), SP (1.5 U/ml) pentosephosphate aldolase, PPA (2 U/ml) and FDP aldolase II (0.5 U/ml) to 10 ml 0.9 M sucrose, pH 7.6, at room temperature.

2 ml of A were added over B at a temperature of 20°C. After 1 hour 2.5 ml A were added. After another hour 3.0 ml A were added. After another 1.5 h 3.5 ml A were added. After another 1.5 h 4 ml A were added and after another 1-1.5 h 5 ml A were added and left to stand overnight.

At each time of addition of A the amounts of FDP, dR5P, dX1P and dA in the reaction mixture were determined and the yield was calculated. The concentration of acetaldehyde was kept between 20-30 mM. The results are shown in Table 1:

Table 1

Time (h)	Volume (ml)	Concentrations (mM)			Yield (mmol)
		dR5P	dA	dX1P	dA
0	12	4	0	1.2	0
1	12	3.4	3.2	1	0.04
2	14.5	7.9	8.0	2.6	0.12
3.5	17.5	13	16.2	4.3	0.28
5	21	11.7	21.7		0.46
6	25		23.7		0.59
22	30	11	40.4	13.2	1.21
30	30		50.3		1.51
54	30	8.9	60.6		1.82

The starting amount of FDP was 1.92 mmol. The amount after completion of reaction was 0.150 mmol. Thus, 1.77 mmol were consumed, theoretically corresponding to 3.54 mmol equivalents dA. The amount of dA formed was 1.82 mmol, leading to a yield of 51.4% based on the amount of FDP.

Example 3

Removal of excess FDP by means of $FeCl_3$

1.4 g (2.55 mmol) trisodium-fructose-1,6-disphosphate-octahydrate and 430 µl (335 mg, 7.6 mmol) acetaldehyde were dissolved in 15 ml of water at 4°C. A pH of 7.9 was adjusted by means of sodium hydroxide solution. 150 U pentosephosphate aldolase (PPA) were added, and cold water (4°C)

was added to give 20 ml. After addition of 50 U E.coli aldolase II the mixture was stored at 4°C. After 2 h another 75 U PPA and 50 µl acetaldehyde (390 mg, 8.9 mmol) were added. After 20 h 500 U triosephosphate isomerase (TIM) were added. After 120 h the solution contained about 68 mM FDP, about 12 mM dX1P and about 45 mM dR5P. The reaction was stopped by adding 900 µl of a.2 M solution of iron(III) chloride in 0.01 M hydrochloric acid. The precipitate was centrifuged and washed, the resulting solution contained about 4 mM dX1P, about 9 mM FDP and about 25 mM dR5P.

Example 4

Removal of excess FDP and dX1P by degradation via DHAP

576 mg (1.05 mmol) trisodium-fructose-1,6-disphosphate-octahydrate were dissolved in 8 ml water, and the pH was adjusted at 8.1 by means of sodium hydroxide solution. 75 U PPA and 27 U rabbit muscle aldolase (RAMA) were added, and water was added to give 10 ml. 570 µl (440 mg, 10 mmol) acetaldehyde were added. The reaction was stored at 4°C. After 100 h the solution contained about 110 mM dX1P, about 5 mM FDP and about 85 mM dR5P (about 870 µmol). The reaction was stopped by adding hydrochloric acid until a pH of 2 was reached. After adding sodium hydroxide solution to give a pH of 5.5 the solution was stored.

For removing dX1P the acetaldehyde was evaporated and the solution was diluted with water to reach 30 ml. It was mixed with 3 ml 2.65 M sodium formate solution (8 mmol), and sodium hydroxide solution was added until a pH of 7.4 was reached. 23 U formate dehydrogenase (FDH), 6 mg NADH, 16 U RAMA and 20 U glycerolphosphate dehydrogenase (GDH) were added.

After 24 h at room temperature the concentrations of dX1P and FDP are below 3 mM, the loss of dR5P is less than 10%.

Example 5

Preparation of dR5P via G3P

1.1 g (2.0 mmol) trisodium-fructose-1,6-disphosphate-octahydrate were dissolved in 8 ml water. 1.58 mol of a 2.65 M sodium formate solution (4.2 mmol) and 14.2 mg NADH were added. A pH of 7.0 was adjusted by means of NaOH. After addition of 36 U RAMA, 50 U triosephosphate isomerase (TIM), 34 U GDH and 35 U FDH water was added to give 12 ml.

After incubation of 40 h at room temperature the FDP content was below 3 mM. The enzymes were denatured by acidification with hydrochloric acid to reach a pH of 2. Subsequently, the pH of the solution was adjusted at 4 and the solids were centrifuged and filtered off, respectively. Through dilution during purification a total volume of 25 ml was reached which contained about 160 mM of glycerol-3-phosphate (G3P).

4 ml of this solution (about 640 µmol G3P) were adjusted at a pH of 7.8 by means of sodium hydroxide solution. 7.8 kU catalase, 500 U TIM and 13 U glycerol 3-phosphate oxidase are added. The mixture was stirred very slowly in an open flask. After 30 min 18 U PPA were added. Acetaldehyde was added in portions of 30 µl (23.5 mg, 530 µmol) after 30, 60, 120, 180 and 240 min. After 24 h another 15 U PPA, 2.5 kU TIM and 100 µl (78 mg, 1.8 mmol) acetaldehyde were added. After 30 h the batch is sealed after addition of another 100 µl acetaldehyde. After a total of 45 h a concentration of about 60 mM dR5P was achieved and the reaction is completed. For preparing 2'-deoxyadenosine (e.g. Example 7) excess acetaldehyde must be distilled off.

Example 6

Preparation of a dR5P solution containing small amounts of dX1P or FDP

A solution of 60 mmol/l FDP and 120 mmol/l acetaldehyde having pH 7.4 was kept at a temperature of 15°C. 5 ml thereof were mixed with 4 U aldolase II, 2 U TIM and 40 U PPA and kept at 15°C. After 4, 8.5, 16.5 and 24 h 12 U PPA and 100 µl of a 34 vol.-% solution of acetaldehyde in water (26.4 mg, 600 µmol) were added each. After 40 h the solution was allowed to reach room temperature. After 90 h the reaction solution had reached concentrations of about 3 mM FDP, about 4 mM dX1P and at least 70 mM dR5P. For stopping the reaction and removing acetaldehyde about 20% of the volume were distilled off.

Example 7

Preparation of deoxyadenosine (dA) from dR5P by means of barium acetate

dR5P was used in the form of a solution prepared according to Examples 3-6. For instance, 10 ml of a solution of Example 6 diluted to have 70 mM dR5P (700 µmol dR5P) were mixed with 40 mg (300 µmol) adenine, 41 µg (50 nmol) tetracyclohexylammonium-glucose-1,6-disphosphate, 396 µg (2 µmol) manganese-II-acetate-tetrahydrate, 10 U pentosephosphate mutase (PPM) and 30 U purine-nucleoside phosphorylase (PNP). After 3 h another 27 mg (200 µmol) adenine and 26 mg (100 µmol) barium acetate were added.

A further amount of 26 mg barium acetate was added after 4 h, one of 40 mg adenine after 7 h. After 10 h the reaction was completed. The solution had a concentration of 45 mM dA.

Example 8

Preparation of deoxyadenosine (dA) from dR5P by means of sucrose phosphorylase

10 ml of a solution of Example 6 diluted to 55 mM dR5P (550 µmol dR5P) were mixed with 81 mg (600 µmol) adenine, 41 µg (50 nmol) tetracyclohexylammonium-glucose-1,6-disphosphate, 396 µg (2 µmol) manganese-II-acetate-tetrahydrate, 10 U pentosephosphate mutase (PPM) 15 U purine nucleoside phosphorylase (PNP), 25 U sucrose phosphorylase and 340 mg (1 mmol) cane sugar.

After 3 h at room temperature the reaction was completed. The solution had a concentration of about 50 mM dA.

Example 9

Preparation of deoxyadenosine (dA) from dR5P by means of maltose phosphorylase

10 ml of a solution of dR5P diluted to 55 mM were mixed at pH 7.0 with 81 mg (600 µmol) adenine, 41 µg (50 nmoles) glucose 1,6-diphosphate, 396 µg (2 µmoles) manganese II-acetate tetrahydrate, 5 units pentose phosphate mutase (PPM), 10 units purine nucleoside phosphorylase, (PNP), 20 units maltose phosphorylase and 1080 mg (3 mmoles) maltose.

After 12h at room temperature the reaction was completed. The solution had a concentration of 49 mM dA.

Example 10

Preparation of deoxycytosine (dC) from dR5P by means of sucrose phosphorylase

20 ml of a solution of dR5P diluted to 70 mM were mixed at pH 7.0 with 5.4 mg adenine (0.04 mmoles), 155 mg cytosine (1.4 mmoles), 82 µg (100 nmoles) glucose 1,6-diphosphate, 792 µg (4 µmoles) manganese II-acetate-tetrahydrate, 20 units PPM, 30 units PNP, 50 units 2-deoxyribosyl transferase (NdT), 50 units sucrose phosphorylase and 2.05 g (6 mmoles) sucrose.

After 18h at 30°C the solution had a concentration of 62 mM dC.

Example 11

Preparation of deoxyguanosine (dG) from dR5P by means of sucrose phosphorylase

20 ml of a solution of dR5P diluted to 70 mM were mixed at pH 7.0 with 91 mg guanine (0.6 mmoles), 82 µg (100 nmoles) glucose 1,6-diphosphate, 792 µg (4 µmoles) manganese II-acetate-tetrahydrate, 20 units PPM, 10 units PNP, 20 units sucrose phosphorylase and 2.05 g (6 mmoles) sucrose.

After 18h at 37°C the dG formed corresponds to 0.5 mmoles.

Example 12

Two step procedure of dA synthesis

In the first step dR5P was prepared by adding FDP-Aldolase II (AldII) from E. coli, pentosephosphate aldolase (PPA) from E. coli and triosephosphate isomerase (TIM) from E. coli to fructose-1.6-bisphosphate (FDP) and acetaldehyde (AcAld) essentially according to Ex. 6. FDP trisodium salt was mixed in a final concentration of 75 mM with AcAld (100 mM final concentration). The pH was adjusted to 7,4 by addition of sodium hydroxide. The reaction was started by adding PPA (12 kU/l), Ald II (0,3 kU/l) and TIM (2,5 kU/l). At 4 h 117 mM AcAld, at 7 h 117 mm AcAld, PPA 6 kU/l, TIM 2,5 kU/l and at 12 h 117 mM AcAld were added. The reaction was run at 21 °C. Conversion was monitored by enzymatical assay using step by step glycerol-3-phosphate dehydrogenase (GDH), rabbit muscle aldolase (RAMA), trisosephosphate isomerase (TIM), pentosephosphate aldolase (PPA) in the presence of NADH (0,26 mM in 300 mM triethanol amine buffer pH 7.6). Conversion is shown in Fig. 1.

After yielding approx. 95 mM dR5P the enzymes were deactivated by heating to 65 °C for 10 min. and excess of AcAld was removed by evaporation. In the second step dR5P in a final concentration of 64 mM was converted to deoxyadenosine (dA) by adding adenine (A, final concentration 58 mM) in the presence of 300 μM $MnCl_2$, 5 μM Glucose-1.6-bisphosphate, pentosephosphate mutase from E. coli (PPM, 2 kU/l), purine nucleoside phosphorylase from E. coli (PNP, 1 kU/l). The synthesis was run at 20 °C, pH 7.4. In one experiment 200 mM sucrose and 0.6 kU/l sucrose phosphorylase (SP) from Leuconostoc mes. were added at t = 2 h (see arrow in Fig. 2, rhombus, solid line), in a second experiment addition of SP was omitted (squares, dotted line). The conversion was monitored by RP-HPLC (column Hypersil ODS 5 μm, 250 x 4,6 mm; eluent: 30 mM potassium phosphate, 5 mM tetrabutyl ammoniumhydrogensulfate pH 6.0/

1 % acetonitrile, flow rate: 1 ml/min, column temp.: 35 °C, det.: UV at 260 nm) and is shown in Fig. 2.

Example 13

dR5P was prepared by adding FDP-Aldolase II (AldII) from E. coli, pentosephosphate aldolase (PPA) from E. coli and trisosephosphate isomerase (TIM) from E. coli to fructose-1.6-bisphosphate (FDP) and acetaldehyde (AcAld) essentially according to Ex. 6. Excess of AcAld was removed by evaporation. dR5P in a final concentration of 60 mM was converted to deoxyadenosine (dA) by adding adenine (A, final concentration 58 mM) in the presence of 300 μM $MnCl_2$, 5 μM Glucose-1.6-bisphosphate, pentosephosphate mutase from E. coli (PPM, 1,5 kU/l), purine nucleoside phosphorylase from E. coli (PNP, 1 kU/l). The synthesis was run at 20 °C, pH 7.4. After 24 h sucrose in a final concentration of 200 mM and sucrose phosphorlyase from Leuconsotoc mes. (1 kU/l) were added. Conversion was monitored by RP-HPLC (dA, A, see ex. 12)) resp. enzymatical assay (dR5P, using step by step glycerol-3-phosphate dehydrogenase (GDH), rabbit muscle aldolase (RAMA), trisosephosphate isomerase (TIM), pentosephosphate aldolase (PPA) in the presence of NADH (0,26 mM in 300 mM Triethanol amine buffer pH 7.6)) and phosphomolybdate complexing of inorg. phosphate (Sigma, Proc. No. 360-UV). This is shown in Fig. 3.

Example 14

dR5P was essentially prepared according according to Ex. 6. dR5P in a final concentration of 80 mM was then converted to deoxy-6-aminoguanosine (dG-NH_2) by adding 2,6-Diaminopurine (DAP, final concentration 77 mM) in the presence of 200 mM sucrose, 300 μM $MnCl_2$, 5 μM Glucose-1.6-bisphosphate, pentosephosphate mutase from E. coli (PPM, 2,5 kU/l), purine nucleoside phosphorylase from E. coli (PNP, 1 kU/l), sucrose

phosphorylase from Leucoonostoc mes. (SP, 1,5 kU/l) . The synthesis was run at 20°C pH 7.4. After 2,5h, 5 h and 20,5 h additional amounts of enzymes were added: 2,5 h PPM (2,5 kU/l), PNP (1 kU/l, SP (1,5 kU/l), 5 h PPM (2,5 kU/l), SP (1,5 kU/l), 20,5 h: PPM (2,5 kU/l), SP (1,5 kU/l). The conversion was monitored by RP-HPLC (column Hypersil ODS 5 µm, 250 x 4,6 mm; eluent: 30 mM potassium phosphate, 5 mM tetrabutyl ammoniumhydrogensulfate pH 6.0/ 1 % acetonitrile, flow rate: 1 ml/min, column temp.: 35°C, det.: UV at 216 nm) and is shown in Fig. 4.

Claims

1. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising reacting deoxyribose 1-phosphate (dR1P) and a nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed.

2. The method of claim 1, wherein the inorganic phosphate is removed.

3. The method of claim 1 or 2, wherein the reaction is catalyzed by a thymidine phosphorylase (TP, EC 2.4.2.4) or a purine nucleoside phosphorylase (PNP, EC 2.4.2.1).

4. The method of any one of the previous claims, wherein the nucleobase is selected from the group consisting of thymine, uracil, adenine, guanine and hypoxanthine and analogs thereof, e.g. 2-thio-uracil, 6-aza-uracil, 5-carboxy-2-thio-uracil, 6-aza-thymine, 6-aza-2-thio-thymine and 2,6-diamino-purine.

5. The method of any one of the previous claims, wherein the removal of the inorganic phosphate is effected by (i) conversion to inorganic pyrophosphate, (ii) precipitation, (iii) complexation and/or (iv) substrate phosphorylation.

6. The method of claim 5, wherein the inorganic phosphate is converted to pyrophosphate by a phosphate transfer from fructose-diphosphate (FDP) under formation of fructose-6-phosphate (F6P).

7. The method of claim 6, wherein the phosphate transfer is catalyzed by a PPi-dependent phosphofructokinase (PFK-PPi, EC 2.7.1.90).

8. The method of claim 6 or 7, wherein the inorganic pyrophosphate is removed by precipitation.

9. The method of claim 5, wherein the inorganic phosphate is transferred to a disaccharide, particularly sucrose or maltose under formation of a monosaccharide and a phosphorylated monosaccharide.

10. The method of claim 9, wherein the phosphate transfer is catalyzed by a sucrose phosphorylase (EC 2.4.1.7) or a maltose phosphorylase (EC 2.4.1.8).

11. The method of claim 10, wherein the phosphorylated monosaccharide is further reacted.

12. The method of any one of the previous claims, wherein the deoxyribose-1-phosphate is generated from deoxyribose 5-phosphate (dR5P).

13. The method of claim 12, wherein the reaction is catalyzed by a deoxyribomutase (EC 2.7.5.1) or a phosphopentose mutase (PPM, EC 5.4.2.7).

14. The method of claim 12 or 13, wherein the deoxyribose-5-phosphate is generated by a condensation of glyceraldehyde 3-phosphate (GAP) with acetaldehyde.

15. The method of claim 14, wherein the reaction is catalyzed by a phosphopentose aldolase (PPA, EC 4.1.2.4).

16. The method of claim 14 or 15, wherein the glyceraldehyde 3-phosphate is generated from fructose 1,6-diphosphate, dihydroxyacetone (DHA) and/or glycerolphosphate.

17. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from fructose 1,6-diphosphate in a reaction catalyzed by an FDP-aldolase (EC 4.1.2.13) selected from FDP-aldolases I and FDP-aldolases II.

18. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from dihydroxyacetone and ATP under formation of dihydroxyacetone phosphate (DHAP) and ADP and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerokinase (GK, EC 2.7.1.30) and a triose phosphate isomerase (TIM, EC 5.3.1.1).

19. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from glycerol phosphate (GP) and O_2 under formation of dihydroxyacetone phosphate (DHAP) and H_2O_2 and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerophosphate oxidase (GPO, EC 1.1.3.21) and a triose phosphate isomerase (TIM, EC 5.3.1.1).

20. The method of claim 12 or 13, wherein the deoxyribose 5-phosphate is generated by a phosphorylation of deoxyribose.

21. The method of claim 20, wherein the reaction is catalyzed by a deoxyribokinase (dRK, EC 2.7.1.15).

22. The method of claim 21, wherein a dRK obtainable from Salmonella typhi is used which is encoded by (a) the nucleotide sequence shown in SEQ ID NO.11 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

23. The method of any one of the previous claims, wherein a deoxyribonucleoside containing a first nucleobase is further reacted with a second nucleobase under formation of a deoxyribonucleoside containing the second nucleobase.

24. The method of claim 23, wherein said second nucleobase is selected from cytidine and analogs thereof, e.g. 5-aza-cytidine, 2,6-dichloro-purine, 6-chloro-guanine, 6-chloro-purine, 6-aza-thymine and 5-fluoro-uracil.

25. The method of claim 24, wherein the reaction is catalyzed by a nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6).

26. The method of claim 25, wherein an NdT obtainable from Lactobacillus leichmannii is used which is encoded by (a) the nucleotide sequence shown in SEQ ID NO.13 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

27. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of:

(i) condensing glyceraldehyde 3-phosphate (GAP) with acetaldehyde to deoxyribose 5-phosphate (dR5P),

(ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate (dR1P) and

(iii) reacting deoxyribose 1-phosphate and nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed.

28. The method of claim 27, wherein the reaction is carried out without isolating intermediate products.

29. The method of claim 27 or 28, wherein the glyceraldehyde 3-phosphate (GAP) is generated from fructose 1,6-diphosphate (FDP), dihydroxy-acetone (DHA) and/or glycerolphosphate (GP).

30. The method of claims 27 to 29, wherein before step (ii) excess acetaldehyde is removed.

31. The method of claims 27 to 30, wherein before step (ii) excess starting materials and/or by-products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate (dX1P) are removed.

32. The method of claims 27 to 30, wherein the reaction is carried out in a manner that before step (ii) no substantial amounts of starting materials and/or by-products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate are present.

33. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of:

(i) phosphorylating deoxyribose to deoxyribose 5-phosphate,

(ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate and

(iii) reacting deoxyribose 1-phosphate and nucleobase, wherein a deoxyribonucleoside an inorganic phosphate are formed.

34. The method of claim 33, wherein the reaction is carried out without isolating intermediate products.

35. The method of claims 27 to 34, wherein the inorganic phosphate is removed.

36. The method of any one of the previous claims comprising further reacting said deoxyribonucleoside.

37. The method of claim 36, wherein said further reacting comprises the synthesis of deoxyribonucleoside mono-, di- or triphosphates, of H-phosphonates or of phosphoramidites.

38. The use of an isolated nucleic acid molecule encoding a nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6) for the preparation of an enzyme for an in vitro method for the enzymatic synthesis of deoxyribonucleosides, wherein a deoxyribonucleoside containing a first nucleobase is further reacted with a second nucleobase under formation of a deoxyribonucleoside containing the second nucleobase, wherein said nucleic acid molecule comprises (a) the nucleotide sequence shown in SEQ ID NO.13 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

39. The use of claim 38, wherein the second nucleobase is selected from cytidine and analogs thereof, e.g. 6-methyl purine, 2-amino-6-methylmercaptopurine, 6-dimethylaminopurine, 5-azacytidine, 2,6-dichloropurine, 6-chloroguanine, 6-chloropurine, 6-azathymine, 5-fluorouracil, ethyl-4-amino-5-imidazole carboxylate, imidazole-4-carboxamide and 1,2,4-triazole-3-carboxamide.

40. The use of claim 38 or 39, wherein the first nucleobase is selected from adenine, guanine, thymine, uracil and hypoxanthine.

41. The use of any one of claims 38-40, wherein the nucleic acid molecule is contained on a recombinant vector in operative linkage with an expression control sequence.

42. The use of any one of claims 38-41, wherein the nucleic acid is contained in a recombinant cell.

43. Use of an isolated polypeptide having NdT activity for the preparation of nucleosides according to claim 24.

44. Use of an isolated nucleic acid molecule encoding a deoxyribokinase (dRK, EC 2.7.1.5) for the preparation of an enzyme for an in vitro method for the enzymatic synthesis of deoxyribonucleosides comprising the step of phosphorylating deoxyribose to deoxyribose 5-phosphate, wherein said nucleic acid molecule comprises (a) the nucleotide sequence shown in SEQ ID NO.11 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

45. Use of an isolated polypeptide having dRK activity for an in vitro method for the enzymatic synthesis of deoxyribonucleosides comprising the step of phosphorylating deoxyribose to deoxyribose 5-phosphate.

46. Recombinant bacteria strains deposited at CNCM under accession numbers I-2186, I-2187, I-2188, I-2189, I-2190 and I-2191.

Fig.1

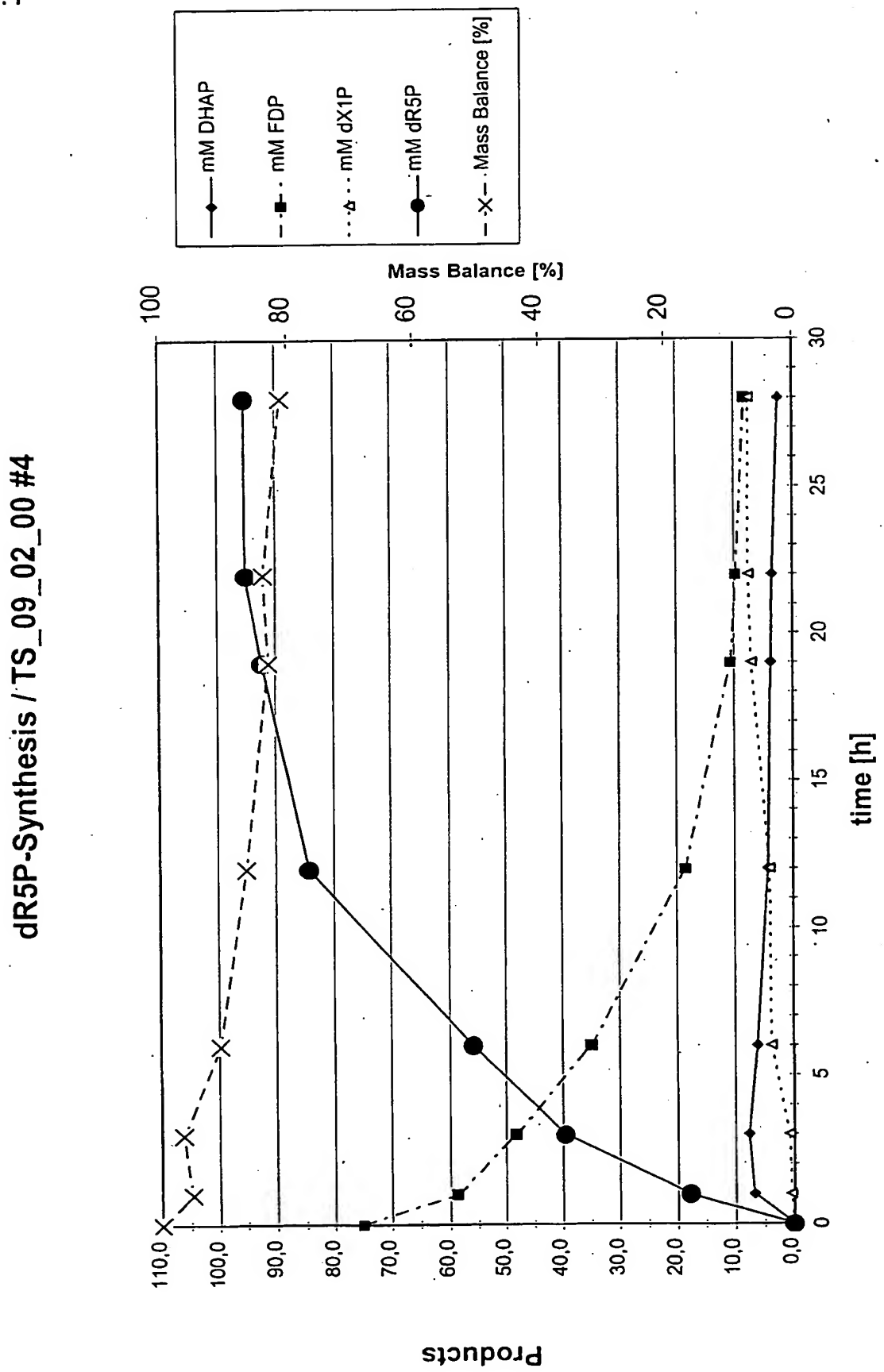

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Fig. 2

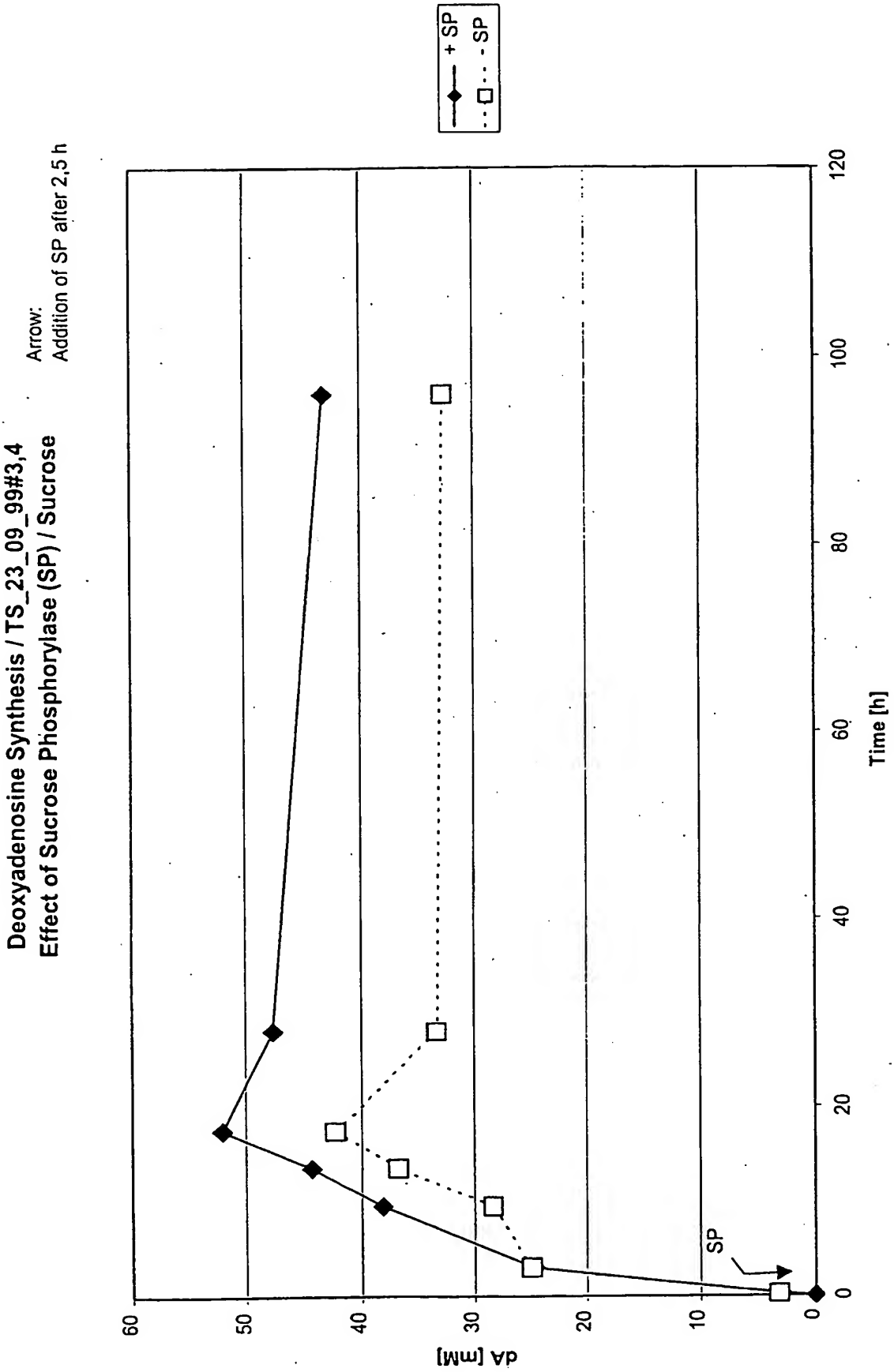
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Fig. 3

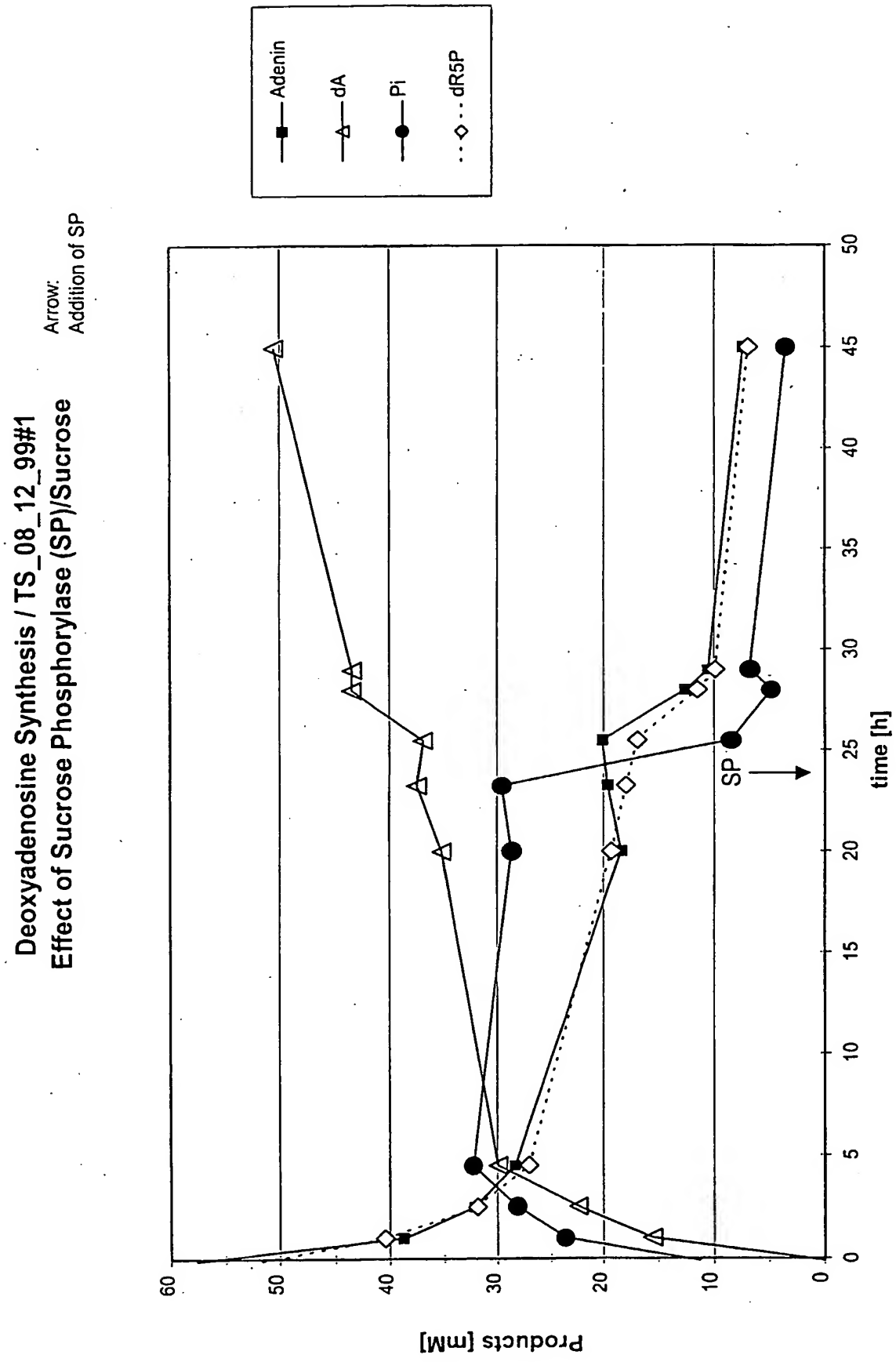
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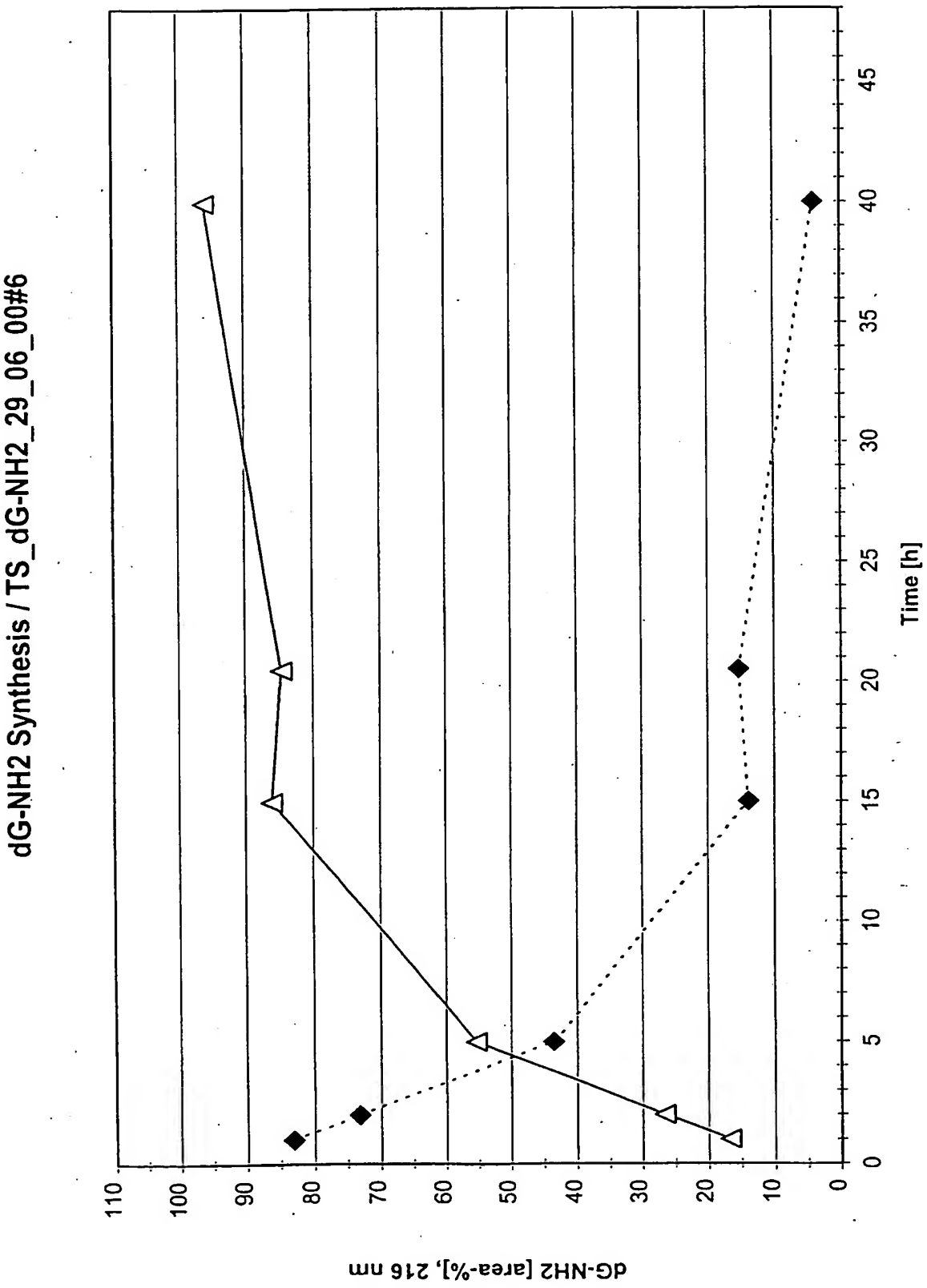
Fig. 4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             20                  25                  30

act atc tcc gaa ggg cag att gcc gcc ctc gcg atg acc att ttc ttc  144
Thr Ile Ser Glu Gly Gln Ile Ala Ala Leu Ala Met Thr Ile Phe Phe
         35                  40                  45
```

```
cac gat atg aca atg cct gag cgt gtc tcg ctg acc atg gcg atg cga   192
His Asp Met Thr Met Pro Glu Arg Val Ser Leu Thr Met Ala Met Arg
     50                  55                  60

gat tca gga acc gtt ctc gac tgg aaa agc ctg cat ctg aat ggc ccg   240
Asp Ser Gly Thr Val Leu Asp Trp Lys Ser Leu His Leu Asn Gly Pro
 65                  70                  75                  80

att gtt gat aaa cac tcc acc ggt ggc gtc ggc gat gtg act tcg ctg   288
Ile Val Asp Lys His Ser Thr Gly Gly Val Gly Asp Val Thr Ser Leu
                 85                  90                  95

atg ttg ggg ccg atg gtc gca gcc tgc ggc ggc tat att ccg atg atc   336
Met Leu Gly Pro Met Val Ala Ala Cys Gly Gly Tyr Ile Pro Met Ile
            100                 105                 110

tct ggt cgc ggc ctc ggt cat act ggc ggt acg ctc gac aaa ctg gaa   384
Ser Gly Arg Gly Leu Gly His Thr Gly Gly Thr Leu Asp Lys Leu Glu
        115                 120                 125

tcc atc cct ggc ttc gac att ttc ccg gat gac aac cgt ttc cgc gaa   432
Ser Ile Pro Gly Phe Asp Ile Phe Pro Asp Asp Asn Arg Phe Arg Glu
    130                 135                 140

att att aaa gac gtc ggc gtg gcg att atc ggt cag acc agt tca ctg   480
Ile Ile Lys Asp Val Gly Val Ala Ile Ile Gly Gln Thr Ser Ser Leu
145                 150                 155                 160

gct ccg gct gat aaa cgt ttc tac gcg acc cgt gat att acc gca acc   528
Ala Pro Ala Asp Lys Arg Phe Tyr Ala Thr Arg Asp Ile Thr Ala Thr
                165                 170                 175

gtg gac tcc atc ccg ctg atc acc gcc tct att ctg gcg aag aaa ctt   576
Val Asp Ser Ile Pro Leu Ile Thr Ala Ser Ile Leu Ala Lys Lys Leu
            180                 185                 190

gcg gaa ggt ctg gac gcg ctg gtg atg gac gtg aaa gtg ggt agc ggc   624
Ala Glu Gly Leu Asp Ala Leu Val Met Asp Val Lys Val Gly Ser Gly
        195                 200                 205

gcg ttt atg ccg acc tac gaa ctc tct gaa gcc ctt gcc gaa gcg att   672
Ala Phe Met Pro Thr Tyr Glu Leu Ser Glu Ala Leu Ala Glu Ala Ile
    210                 215                 220
```

```
gtt ggc gtg gct aac ggc gct ggc gtg cgc acc acc gcg ctg ctc acc   720
Val Gly Val Ala Asn Gly Ala Gly Val Arg Thr Thr Ala Leu Leu Thr
225                 230                 235                 240

gac atg aat cag gta ctg gcc tcc agt gca ggt aac gcg gtt gaa gtt   768
Asp Met Asn Gln Val Leu Ala Ser Ser Ala Gly Asn Ala Val Glu Val
                245                 250                 255

cgt gaa gcg gtg cag ttc ctg acg ggt gaa tat cgt aac ccg cgt ctg   816
Arg Glu Ala Val Gln Phe Leu Thr Gly Glu Tyr Arg Asn Pro Arg Leu
            260                 265                 270

ttt gat gtc acg atg gcg ctg tgc gtg gag atg ctg atc tcc ggc aaa   864
Phe Asp Val Thr Met Ala Leu Cys Val Glu Met Leu Ile Ser Gly Lys
        275                 280                 285

ctg gcg aaa gat gac gcc gaa gcg cgc gcg aaa ttg cag gcg gtg ctg   912
Leu Ala Lys Asp Asp Ala Glu Ala Arg Ala Lys Leu Gln Ala Val Leu
    290                 295                 300

gac aac ggt aaa gcg gca gaa gtc ttt ggt cgt atg gta gcg gca caa   960
Asp Asn Gly Lys Ala Ala Glu Val Phe Gly Arg Met Val Ala Ala Gln
305                 310                 315                 320

aaa ggc ccg acc gac ttc gtt gag aac tac gcg aag tat ctg ccg aca   1008
Lys Gly Pro Thr Asp Phe Val Glu Asn Tyr Ala Lys Tyr Leu Pro Thr
                325                 330                 335

gcg atg ctg acg aaa gca gtc tat gct gat acc gaa ggt ttt gtc agt   1056
Ala Met Leu Thr Lys Ala Val Tyr Ala Asp Thr Glu Gly Phe Val Ser
            340                 345                 350

gaa atg gat acc cgc gcg ctg ggg atg gca gtg gtt gca atg ggc ggc   1104
Glu Met Asp Thr Arg Ala Leu Gly Met Ala Val Val Ala Met Gly Gly
        355                 360                 365

gga cgc cgt cag gca tct gac acc atc gat tac agc gtc ggc ttt act   1152
Gly Arg Arg Gln Ala Ser Asp Thr Ile Asp Tyr Ser Val Gly Phe Thr
    370                 375                 380

gat atg gcg cgt ctg ggc gac cag gta gac ggt cag cgt ccg ctg gcg   1200
Asp Met Ala Arg Leu Gly Asp Gln Val Asp Gly Gln Arg Pro Leu Ala
385                 390                 395                 400
```

```
gtt atc cac gcg aaa gac gaa aac aac tgg cag gaa gcg gcg aaa gcg   1248
Val Ile His Ala Lys Asp Glu Asn Asn Trp Gln Glu Ala Ala Lys Ala
                405                 410                 415

gtg aaa gcg gca att aaa ctt gcc gat aaa gca ccg gaa agc aca cca   1296
Val Lys Ala Ala Ile Lys Leu Ala Asp Lys Ala Pro Glu Ser Thr Pro
            420                 425                 430

act gtc tat cgc cgt atc agc gaa taa                              1323
Thr Val Tyr Arg Arg Ile Ser Glu
        435                 440
```

<210> 2
<211> 440
<212> PRT
<213> Escherichia coli

<400> 2

```
Leu Phe Leu Ala Gln Glu Ile Ile Arg Lys Lys Arg Asp Gly His Ala
  1               5                  10                  15

Leu Ser Asp Glu Glu Ile Arg Phe Phe Ile Asn Gly Ile Arg Asp Asn
             20                  25                  30

Thr Ile Ser Glu Gly Gln Ile Ala Ala Leu Ala Met Thr Ile Phe Phe
         35                  40                  45

His Asp Met Thr Met Pro Glu Arg Val Ser Leu Thr Met Ala Met Arg
     50                  55                  60

Asp Ser Gly Thr Val Leu Asp Trp Lys Ser Leu His Leu Asn Gly Pro
 65                  70                  75                  80

Ile Val Asp Lys His Ser Thr Gly Gly Val Gly Asp Val Thr Ser Leu
                 85                  90                  95

Met Leu Gly Pro Met Val Ala Ala Cys Gly Gly Tyr Ile Pro Met Ile
            100                 105                 110

Ser Gly Arg Gly Leu Gly His Thr Gly Gly Thr Leu Asp Lys Leu Glu
        115                 120                 125

Ser Ile Pro Gly Phe Asp Ile Phe Pro Asp Asp Asn Arg Phe Arg Glu
    130                 135                 140
```

Ile Ile Lys Asp Val Gly Val Ala Ile Ile Gly Gln Thr Ser Ser Leu
145 150 155 160

Ala Pro Ala Asp Lys Arg Phe Tyr Ala Thr Arg Asp Ile Thr Ala Thr
165 170 175

Val Asp Ser Ile Pro Leu Ile Thr Ala Ser Ile Leu Ala Lys Lys Leu
180 185 190

Ala Glu Gly Leu Asp Ala Leu Val Met Asp Val Lys Val Gly Ser Gly
195 200 205

Ala Phe Met Pro Thr Tyr Glu Leu Ser Glu Ala Leu Ala Glu Ala Ile
210 215 220

Val Gly Val Ala Asn Gly Ala Gly Val Arg Thr Thr Ala Leu Leu Thr
225 230 235 240

Asp Met Asn Gln Val Leu Ala Ser Ser Ala Gly Asn Ala Val Glu Val
245 250 255

Arg Glu Ala Val Gln Phe Leu Thr Gly Glu Tyr Arg Asn Pro Arg Leu
260 265 270

Phe Asp Val Thr Met Ala Leu Cys Val Glu Met Leu Ile Ser Gly Lys
275 280 285

Leu Ala Lys Asp Asp Ala Glu Ala Arg Ala Lys Leu Gln Ala Val Leu
290 295 300

Asp Asn Gly Lys Ala Ala Glu Val Phe Gly Arg Met Val Ala Ala Gln
305 310 315 320

Lys Gly Pro Thr Asp Phe Val Glu Asn Tyr Ala Lys Tyr Leu Pro Thr
325 330 335

Ala Met Leu Thr Lys Ala Val Tyr Ala Asp Thr Glu Gly Phe Val Ser
340 345 350

Glu Met Asp Thr Arg Ala Leu Gly Met Ala Val Val Ala Met Gly Gly
355 360 365

Gly Arg Arg Gln Ala Ser Asp Thr Ile Asp Tyr Ser Val Gly Phe Thr
370 375 380

```
Asp Met Ala Arg Leu Gly Asp Gln Val Asp Gly Gln Arg Pro Leu Ala
385                 390                 395                 400

Val Ile His Ala Lys Asp Glu Asn Asn Trp Gln Glu Ala Ala Lys Ala
                405                 410                 415

Val Lys Ala Ala Ile Lys Leu Ala Asp Lys Ala Pro Glu Ser Thr Pro
            420                 425                 430

Thr Val Tyr Arg Arg Ile Ser Glu
        435                 440
```

<210> 3
<211> 720
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(717)

<400> 3

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atg gct acc cca cac att aat gca gaa atg ggc gat ttc gct gac gta   48
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
  1               5                  10                  15

gtt ttg atg cca ggc gac ccg ctg cgt gcg aag tat att gct gaa act   96
Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
             20                  25                  30

ttc ctt gaa gat gcc cgt gaa gtg aac aac gtt cgc ggt atg ctg ggc   144
Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
         35                  40                  45

ttc acc ggt act tac aaa ggc cgc aaa att tcc gta atg ggt cac ggt   192
Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
     50                  55                  60

atg ggt atc ccg tcc tgc tcc atc tac acc aaa gaa ctg atc acc gat   240
Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
 65                  70                  75                  80
```

```
ttc ggc gtg aag aaa att atc cgc gtg ggt tcc tgt ggc gca gtt ctg   288
Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
                85                  90                  95

ccg cac gta aaa ctg cgc gac gtc gtt atc ggt atg ggt gcc tgc acc   336
Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Ala Cys Thr
            100                 105                 110

gat tcc aaa gtt aac cgc atc cgt ttt aaa gac cat gac ttt gcc gct   384
Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125

atc gct gac ttc gac atg gtg cgt aac gca gta gat gca gct aaa gca   432
Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

ctg ggt att gat gct cgc gtg ggt aac ctg ttc tcc gct gac ctg ttc   480
Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

tac tct ccg gac ggc gaa atg ttc gac gtg atg gaa aaa tac ggc att   528
Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175

ctc ggc gtg gaa atg gaa gcg gct ggt atc tac ggc gtc gct gca gaa   576
Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

ttt ggc gcg aaa gcc ctg acc atc tgc acc gta tct gac cac atc cgc   624
Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

act cac gag cag acc act gcc gct gag cgt cag act acc ttc aac gac   672
Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asp
    210                 215                 220

atg atc aaa atc gca ctg gaa tcc gtt ctg ctg ggc gat aaa gag taa   720
Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 4
<211> 239
<212> PRT
<213> Escherichia coli

<400> 4

```
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
 1               5                  10                  15

Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
            20                  25                  30

Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
        35                  40                  45

Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
    50                  55                  60

Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
65                  70                  75                  80

Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
                85                  90                  95

Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Ala Cys Thr
            100                 105                 110

Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125

Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175

Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asp
    210                 215                 220

Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 5
<211> 1224
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(1221)

<400> 5

```
atg aaa cgt gca ttt att atg gtg ctg gac tca ttc ggc atc ggc gct   48
Met Lys Arg Ala Phe Ile Met Val Leu Asp Ser Phe Gly Ile Gly Ala
 1               5                  10                  15

aca gaa gat gca gaa cgc ttt ggt gac gtc ggg gct gac acc ctg ggt   96
Thr Glu Asp Ala Glu Arg Phe Gly Asp Val Gly Ala Asp Thr Leu Gly
            20                  25                  30

cat atc gca gaa gct tgt gcc aaa ggc gaa gct gat aac ggt cgt aaa   144
His Ile Ala Glu Ala Cys Ala Lys Gly Glu Ala Asp Asn Gly Arg Lys
        35                  40                  45

ggc ccg ctc aat ctg cca aat ctg acc cgt ctg ggg ctg gcg aaa gca   192
Gly Pro Leu Asn Leu Pro Asn Leu Thr Arg Leu Gly Leu Ala Lys Ala
    50                  55                  60

cac gaa ggt tct acc ggt ttc att ccg gcg gga atg gac ggc aac gct   240
His Glu Gly Ser Thr Gly Phe Ile Pro Ala Gly Met Asp Gly Asn Ala
65                  70                  75                  80

gaa gtt atc ggc gcg tac gca tgg gcg cac gaa atg tca tcc ggt aaa   288
Glu Val Ile Gly Ala Tyr Ala Trp Ala His Glu Met Ser Ser Gly Lys
                85                  90                  95

gat acc ccg tct ggt cac tgg gaa att gcc ggt gtc ccg gtt ctg ttt   336
Asp Thr Pro Ser Gly His Trp Glu Ile Ala Gly Val Pro Val Leu Phe
            100                 105                 110

gag tgg gga tat ttc tcc gat cac gaa aac agc ttc ccg caa gag ctg   384
Glu Trp Gly Tyr Phe Ser Asp His Glu Asn Ser Phe Pro Gln Glu Leu
        115                 120                 125
```

```
ctg gat aaa ctg gtc gaa cgc gct aat ctg ccg ggt tac ctc ggt aac   432
Leu Asp Lys Leu Val Glu Arg Ala Asn Leu Pro Gly Tyr Leu Gly Asn
    130                 135                 140

tgc cac tct tcc ggt acg gtc att ctg gat caa ctg ggc gaa gag cac   480
Cys His Ser Ser Gly Thr Val Ile Leu Asp Gln Leu Gly Glu Glu His
145                 150                 155                 160

atg aaa acc ggc aag ccg att ttc tat acc tcc gct gac tcc gtg ttc   528
Met Lys Thr Gly Lys Pro Ile Phe Tyr Thr Ser Ala Asp Ser Val Phe
                165                 170                 175

cag att gcc tgc cat gaa gaa act ttc ggt ctg gat aaa ctc tac gaa   576
Gln Ile Ala Cys His Glu Glu Thr Phe Gly Leu Asp Lys Leu Tyr Glu
            180                 185                 190

ctg tgc gaa atc gcc cgt gaa gag ctg acc aac ggc ggc tac aat atc   624
Leu Cys Glu Ile Ala Arg Glu Glu Leu Thr Asn Gly Gly Tyr Asn Ile
        195                 200                 205

ggt cgt gtt atc gct cgt ccg ttt atc ggc gac aaa gcc ggt aac ttc   672
Gly Arg Val Ile Ala Arg Pro Phe Ile Gly Asp Lys Ala Gly Asn Phe
    210                 215                 220

cag cgt acc ggt aac cgt cac gac ctg gct gtt gag ccg cca gca ccg   720
Gln Arg Thr Gly Asn Arg His Asp Leu Ala Val Glu Pro Pro Ala Pro
225                 230                 235                 240

acc gtg ctg cag aaa ctg gtt gat gaa aaa cac ggc cag gtg gtt tct   768
Thr Val Leu Gln Lys Leu Val Asp Glu Lys His Gly Gln Val Val Ser
                245                 250                 255

gtc ggt aaa att gcg gac atc tac gcc aac tgc ggt atc acc aaa aaa   816
Val Gly Lys Ile Ala Asp Ile Tyr Ala Asn Cys Gly Ile Thr Lys Lys
            260                 265                 270

gtg aaa gcg act ggc ctg gac gcg ctg ttt gac gcc acc atc aaa gag   864
Val Lys Ala Thr Gly Leu Asp Ala Leu Phe Asp Ala Thr Ile Lys Glu
        275                 280                 285

atg aaa gaa gcg ggt gat aac acc atc gtc ttc acc aac ttc gtt gac   912
Met Lys Glu Ala Gly Asp Asn Thr Ile Val Phe Thr Asn Phe Val Asp
    290                 295                 300
```

```
ttc gac tct tcc tgg ggc cac cgt cgc gac gtc gcc ggt tat gcc gcg   960
Phe Asp Ser Ser Trp Gly His Arg Arg Asp Val Ala Gly Tyr Ala Ala
305                 310                 315                 320

ggt ctg gaa ctg ttc gac cgc cgt ctg ccg gag ctg atg tct ctg ctg   1008
Gly Leu Glu Leu Phe Asp Arg Arg Leu Pro Glu Leu Met Ser Leu Leu
                325                 330                 335

cgc gat gac gac atc ctg atc ctc acc gct gac cac ggt tgc gat ccg   1056
Arg Asp Asp Asp Ile Leu Ile Leu Thr Ala Asp His Gly Cys Asp Pro
            340                 345                 350

acc tgg acc ggt act gac cac acg cgt gaa cac att ccg gta ctg gta   1104
Thr Trp Thr Gly Thr Asp His Thr Arg Glu His Ile Pro Val Leu Val
        355                 360                 365

tat ggc ccg aaa gta aaa ccg ggc tca ctg ggt cat cgt gaa acc ttc   1152
Tyr Gly Pro Lys Val Lys Pro Gly Ser Leu Gly His Arg Glu Thr Phe
    370                 375                 380

gcg gat atc ggc cag act ctg gca aaa tat ttt ggt act tct gat atg   1200
Ala Asp Ile Gly Gln Thr Leu Ala Lys Tyr Phe Gly Thr Ser Asp Met
385                 390                 395                 400

gaa tat ggc aaa gcc atg ttc tga                                   1224
Glu Tyr Gly Lys Ala Met Phe
                405
```

<210> 6
<211> 407
<212> PRT
<213> Escherichia coli

<400> 6

```
Met Lys Arg Ala Phe Ile Met Val Leu Asp Ser Phe Gly Ile Gly Ala
  1               5                  10                  15

Thr Glu Asp Ala Glu Arg Phe Gly Asp Val Gly Ala Asp Thr Leu Gly
            20                  25                  30

His Ile Ala Glu Ala Cys Ala Lys Gly Glu Ala Asp Asn Gly Arg Lys
        35                  40                  45
```

```
Gly Pro Leu Asn Leu Pro Asn Leu Thr Arg Leu Gly Leu Ala Lys Ala
     50                  55                  60

His Glu Gly Ser Thr Gly Phe Ile Pro Ala Gly Met Asp Gly Asn Ala
 65                  70                  75                  80

Glu Val Ile Gly Ala Tyr Ala Trp Ala His Glu Met Ser Ser Gly Lys
                 85                  90                  95

Asp Thr Pro Ser Gly His Trp Glu Ile Ala Gly Val Pro Val Leu Phe
            100                 105                 110

Glu Trp Gly Tyr Phe Ser Asp His Glu Asn Ser Phe Pro Gln Glu Leu
        115                 120                 125

Leu Asp Lys Leu Val Glu Arg Ala Asn Leu Pro Gly Tyr Leu Gly Asn
    130                 135                 140

Cys His Ser Ser Gly Thr Val Ile Leu Asp Gln Leu Gly Glu Glu His
145                 150                 155                 160

Met Lys Thr Gly Lys Pro Ile Phe Tyr Thr Ser Ala Asp Ser Val Phe
                165                 170                 175

Gln Ile Ala Cys His Glu Glu Thr Phe Gly Leu Asp Lys Leu Tyr Glu
            180                 185                 190

Leu Cys Glu Ile Ala Arg Glu Glu Leu Thr Asn Gly Gly Tyr Asn Ile
        195                 200                 205

Gly Arg Val Ile Ala Arg Pro Phe Ile Gly Asp Lys Ala Gly Asn Phe
    210                 215                 220

Gln Arg Thr Gly Asn Arg His Asp Leu Ala Val Glu Pro Pro Ala Pro
225                 230                 235                 240

Thr Val Leu Gln Lys Leu Val Asp Glu Lys His Gly Gln Val Val Ser
                245                 250                 255

Val Gly Lys Ile Ala Asp Ile Tyr Ala Asn Cys Gly Ile Thr Lys Lys
            260                 265                 270

Val Lys Ala Thr Gly Leu Asp Ala Leu Phe Asp Ala Thr Ile Lys Glu
        275                 280                 285
```

```
Met Lys Glu Ala Gly Asp Asn Thr Ile Val Phe Thr Asn Phe Val Asp
    290                 295                 300

Phe Asp Ser Ser Trp Gly His Arg Arg Asp Val Ala Gly Tyr Ala Ala
305                 310                 315                 320

Gly Leu Glu Leu Phe Asp Arg Arg Leu Pro Glu Leu Met Ser Leu Leu
                325                 330                 335

Arg Asp Asp Asp Ile Leu Ile Leu Thr Ala Asp His Gly Cys Asp Pro
            340                 345                 350

Thr Trp Thr Gly Thr Asp His Thr Arg Glu His Ile Pro Val Leu Val
        355                 360                 365

Tyr Gly Pro Lys Val Lys Pro Gly Ser Leu Gly His Arg Glu Thr Phe
    370                 375                 380

Ala Asp Ile Gly Gln Thr Leu Ala Lys Tyr Phe Gly Thr Ser Asp Met
385                 390                 395                 400

Glu Tyr Gly Lys Ala Met Phe
                405
```

<210> 7
<211> 780
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(777)

<400> 7

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atg act gat ctg aaa gca agc agc ctg cgt gca ctg aaa ttg atg gac   48
Met Thr Asp Leu Lys Ala Ser Ser Leu Arg Ala Leu Lys Leu Met Asp
  1               5                  10                  15

ctg aac acc ctg aat gac gac gac acc gac gag aaa gtg atc gcc ctg   96
Leu Asn Thr Leu Asn Asp Asp Asp Thr Asp Glu Lys Val Ile Ala Leu
            20                  25                  30
```

```
tgt cat cag gcc aaa act ccg gtc ggc aat acc gcc gct atc tgt atc   144
Cys His Gln Ala Lys Thr Pro Val Gly Asn Thr Ala Ala Ile Cys Ile
         35                  40                  45

tat cct cgc ttt atc ccg att gct cgc aaa act ctg aaa gag cag ggc   192
Tyr Pro Arg Phe Ile Pro Ile Ala Arg Lys Thr Leu Lys Glu Gln Gly
     50                  55                  60

acc ccg gaa atc cgt atc gct acg gta acc aac ttc cca cac ggt aac   240
Thr Pro Glu Ile Arg Ile Ala Thr Val Thr Asn Phe Pro His Gly Asn
 65                  70                  75                  80

gac gac atc gac atc gcg ctg gca gaa acc cgt gcg gca atc gcc tac   288
Asp Asp Ile Asp Ile Ala Leu Ala Glu Thr Arg Ala Ala Ile Ala Tyr
                 85                  90                  95

ggt gct gat gaa gtt gac gtt gtg ttc ccg tac cgc gcg ctg atg gcg   336
Gly Ala Asp Glu Val Asp Val Val Phe Pro Tyr Arg Ala Leu Met Ala
            100                 105                 110

ggt aac gag cag gtt ggt ttt gac ctg gtg aaa gcc tgt aaa gag gct   384
Gly Asn Glu Gln Val Gly Phe Asp Leu Val Lys Ala Cys Lys Glu Ala
        115                 120                 125

tgc gcg gca gcg aat gta ctg ctg aaa gtg atc atc gaa acc ggc gaa   432
Cys Ala Ala Ala Asn Val Leu Leu Lys Val Ile Ile Glu Thr Gly Glu
    130                 135                 140

ctg aaa gac gaa gcg ctg atc cgt aaa gcg tct gaa atc tcc atc aaa   480
Leu Lys Asp Glu Ala Leu Ile Arg Lys Ala Ser Glu Ile Ser Ile Lys
145                 150                 155                 160

gcg ggt gcg gac ttc atc aaa acc tct acc ggt aaa gtg gct gtg aac   528
Ala Gly Ala Asp Phe Ile Lys Thr Ser Thr Gly Lys Val Ala Val Asn
                165                 170                 175

gcg acg ccg gaa agc gcg cgc atc atg atg gaa gtg atc cgt gat atg   576
Ala Thr Pro Glu Ser Ala Arg Ile Met Met Glu Val Ile Arg Asp Met
            180                 185                 190

ggc gta gaa aaa acc gtt ggt ttc aaa ccg gcg ggc ggc gtg cgt act   624
Gly Val Glu Lys Thr Val Gly Phe Lys Pro Ala Gly Gly Val Arg Thr
        195                 200                 205
```

```
gcg gaa gat gcg cag aaa tat ctc gcc att gca gat gaa ctg ttc ggt   672
Ala Glu Asp Ala Gln Lys Tyr Leu Ala Ile Ala Asp Glu Leu Phe Gly
    210                 215                 220

gct gac tgg gca gat gcg cgt cac tac cgc ttt ggc gct tcc agc ctg   720
Ala Asp Trp Ala Asp Ala Arg His Tyr Arg Phe Gly Ala Ser Ser Leu
225                 230                 235                 240

ctg gca agc ctg ctg aaa gcg ctg ggt cac ggc gac ggt aag agc gcc   768
Leu Ala Ser Leu Leu Lys Ala Leu Gly His Gly Asp Gly Lys Ser Ala
                245                 250                 255

agc agc tac taa                                                  780
Ser Ser Tyr
```

<210> 8
<211> 259
<212> PRT
<213> Escherichia coli

<400> 8

```
Met Thr Asp Leu Lys Ala Ser Ser Leu Arg Ala Leu Lys Leu Met Asp
 1               5                  10                  15

Leu Asn Thr Leu Asn Asp Asp Asp Thr Asp Glu Lys Val Ile Ala Leu
            20                  25                  30

Cys His Gln Ala Lys Thr Pro Val Gly Asn Thr Ala Ala Ile Cys Ile
        35                  40                  45

Tyr Pro Arg Phe Ile Pro Ile Ala Arg Lys Thr Leu Lys Glu Gln Gly
    50                  55                  60

Thr Pro Glu Ile Arg Ile Ala Thr Val Thr Asn Phe Pro His Gly Asn
65                  70                  75                  80

Asp Asp Ile Asp Ile Ala Leu Ala Glu Thr Arg Ala Ala Ile Ala Tyr
                85                  90                  95

Gly Ala Asp Glu Val Asp Val Val Phe Pro Tyr Arg Ala Leu Met Ala
            100                 105                 110

Gly Asn Glu Gln Val Gly Phe Asp Leu Val Lys Ala Cys Lys Glu Ala
        115                 120                 125
```

```
Cys Ala Ala Ala Asn Val Leu Leu Lys Val Ile Ile Glu Thr Gly Glu
    130                 135                 140

Leu Lys Asp Glu Ala Leu Ile Arg Lys Ala Ser Glu Ile Ser Ile Lys
145                 150                 155                 160

Ala Gly Ala Asp Phe Ile Lys Thr Ser Thr Gly Lys Val Ala Val Asn
                165                 170                 175

Ala Thr Pro Glu Ser Ala Arg Ile Met Met Glu Val Ile Arg Asp Met
            180                 185                 190

Gly Val Glu Lys Thr Val Gly Phe Lys Pro Ala Gly Gly Val Arg Thr
        195                 200                 205

Ala Glu Asp Ala Gln Lys Tyr Leu Ala Ile Ala Asp Glu Leu Phe Gly
    210                 215                 220

Ala Asp Trp Ala Asp Ala Arg His Tyr Arg Phe Gly Ala Ser Ser Leu
225                 230                 235                 240

Leu Ala Ser Leu Leu Lys Ala Leu Gly His Gly Asp Gly Lys Ser Ala
                245                 250                 255

Ser Ser Tyr
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<210> 9
<211> 1080
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(1077)

<400> 9

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atg tct aag att ttt gat ttc gta aaa cct ggc gta atc act ggt gat   48
Met Ser Lys Ile Phe Asp Phe Val Lys Pro Gly Val Ile Thr Gly Asp
  1               5                  10                  15
```

```
gac gta cag aaa gtt ttc cag gta gca aaa gaa aac aac ttc gca ctg   96
Asp Val Gln Lys Val Phe Gln Val Ala Lys Glu Asn Asn Phe Ala Leu
            20                  25                  30

cca gca gta aac tgc gtc ggt act gac tcc atc aac gcc gta ctg gaa  144
Pro Ala Val Asn Cys Val Gly Thr Asp Ser Ile Asn Ala Val Leu Glu
        35                  40                  45

acc gct gct aaa gtt aaa gcg ccg gtt atc gtt cag ttc tcc aac ggt  192
Thr Ala Ala Lys Val Lys Ala Pro Val Ile Val Gln Phe Ser Asn Gly
    50                  55                  60

ggt gct tcc ttt atc gct ggt aaa ggc gtg aaa tct gac gtt ccg cag  240
Gly Ala Ser Phe Ile Ala Gly Lys Gly Val Lys Ser Asp Val Pro Gln
65                  70                  75                  80

ggt gct gct atc ctg ggc gcg atc tct ggt gcg cat cac gtt cac cag  288
Gly Ala Ala Ile Leu Gly Ala Ile Ser Gly Ala His His Val His Gln
                85                  90                  95

atg gct gaa cat tat ggt gtt ccg gtt atc ctg cac act gac cac tgc  336
Met Ala Glu His Tyr Gly Val Pro Val Ile Leu His Thr Asp His Cys
            100                 105                 110

gcg aag aaa ctg ctg ccg tgg atc gac ggt ctg ttg gac gcg ggt gaa  384
Ala Lys Lys Leu Leu Pro Trp Ile Asp Gly Leu Leu Asp Ala Gly Glu
        115                 120                 125

aaa cac ttc gca gct acc ggt aag ccg ctg ttc tct tct cac atg atc  432
Lys His Phe Ala Ala Thr Gly Lys Pro Leu Phe Ser Ser His Met Ile
    130                 135                 140

gac ctg tct gaa gaa tct ctg caa gag aac atc gaa atc tgc tct aaa  480
Asp Leu Ser Glu Glu Ser Leu Gln Glu Asn Ile Glu Ile Cys Ser Lys
145                 150                 155                 160

tac ctg gag cgc atg tcc aaa atc ggc atg act ctg gaa atc gaa ctg  528
Tyr Leu Glu Arg Met Ser Lys Ile Gly Met Thr Leu Glu Ile Glu Leu
                165                 170                 175

ggt tgc acc ggt ggt gaa gaa gac ggc gtg gac aac agc cac atg gac  576
Gly Cys Thr Gly Gly Glu Glu Asp Gly Val Asp Asn Ser His Met Asp
            180                 185                 190
```

```
gct tct gca ctg tac acc cag ccg gaa gac gtt gat tac gca tac acc   624
Ala Ser Ala Leu Tyr Thr Gln Pro Glu Asp Val Asp Tyr Ala Tyr Thr
        195                 200                 205

gaa ctg agc aaa atc agc ccg cgt ttc acc atc gca gcg tcc ttc ggt   672
Glu Leu Ser Lys Ile Ser Pro Arg Phe Thr Ile Ala Ala Ser Phe Gly
    210                 215                 220

aac gta cac ggt gtt tac aag ccg ggt aac gtg gtt ctg act ccg acc   720
Asn Val His Gly Val Tyr Lys Pro Gly Asn Val Val Leu Thr Pro Thr
225                 230                 235                 240

atc ctg cgt gat tct cag gaa tat gtt tcc aag aaa cac aac ctg ccg   768
Ile Leu Arg Asp Ser Gln Glu Tyr Val Ser Lys Lys His Asn Leu Pro
                245                 250                 255

cac aac agc ctg aac ttc gta ttc cac ggt ggt tcc ggt tct act gct   816
His Asn Ser Leu Asn Phe Val Phe His Gly Gly Ser Gly Ser Thr Ala
            260                 265                 270

cag gaa atc aaa gac tcc gta agc tac ggc gta gta aaa atg aac atc   864
Gln Glu Ile Lys Asp Ser Val Ser Tyr Gly Val Val Lys Met Asn Ile
        275                 280                 285

gat acc gat acc caa tgg gca acc tgg gaa ggc gtt ctg aac tac tac   912
Asp Thr Asp Thr Gln Trp Ala Thr Trp Glu Gly Val Leu Asn Tyr Tyr
    290                 295                 300

aaa gcg aac gaa gct tat ctg cag ggt cag ctg ggt aac ccg aaa ggc   960
Lys Ala Asn Glu Ala Tyr Leu Gln Gly Gln Leu Gly Asn Pro Lys Gly
305                 310                 315                 320

gaa gat cag ccg aac aag aaa tac tac gat ccg cgc gta tgg ctg cgt   1008
Glu Asp Gln Pro Asn Lys Lys Tyr Tyr Asp Pro Arg Val Trp Leu Arg
                325                 330                 335

gcc ggt cag act tcg atg atc gct cgt ctg gag aaa gca ttc cag gaa   1056
Ala Gly Gln Thr Ser Met Ile Ala Arg Leu Glu Lys Ala Phe Gln Glu
            340                 345                 350

ctg aac gcg atc gac gtt ctg taa                                  1080
Leu Asn Ala Ile Asp Val Leu
        355
```

<210> 10
<211> 359
<212> PRT
<213> Escherichia coli

<400> 10

```
Met Ser Lys Ile Phe Asp Phe Val Lys Pro Gly Val Ile Thr Gly Asp
 1               5                  10                  15

Asp Val Gln Lys Val Phe Gln Val Ala Lys Glu Asn Asn Phe Ala Leu
            20                  25                  30

Pro Ala Val Asn Cys Val Gly Thr Asp Ser Ile Asn Ala Val Leu Glu
        35                  40                  45

Thr Ala Ala Lys Val Lys Ala Pro Val Ile Val Gln Phe Ser Asn Gly
    50                  55                  60

Gly Ala Ser Phe Ile Ala Gly Lys Gly Val Lys Ser Asp Val Pro Gln
65                  70                  75                  80

Gly Ala Ala Ile Leu Gly Ala Ile Ser Gly Ala His His Val His Gln
                85                  90                  95

Met Ala Glu His Tyr Gly Val Pro Val Ile Leu His Thr Asp His Cys
            100                 105                 110

Ala Lys Lys Leu Leu Pro Trp Ile Asp Gly Leu Leu Asp Ala Gly Glu
        115                 120                 125

Lys His Phe Ala Ala Thr Gly Lys Pro Leu Phe Ser Ser His Met Ile
    130                 135                 140

Asp Leu Ser Glu Glu Ser Leu Gln Glu Asn Ile Glu Ile Cys Ser Lys
145                 150                 155                 160

Tyr Leu Glu Arg Met Ser Lys Ile Gly Met Thr Leu Glu Ile Glu Leu
                165                 170                 175

Gly Cys Thr Gly Gly Glu Glu Asp Gly Val Asp Asn Ser His Met Asp
            180                 185                 190

Ala Ser Ala Leu Tyr Thr Gln Pro Glu Asp Val Asp Tyr Ala Tyr Thr
        195                 200                 205
```

```
Glu Leu Ser Lys Ile Ser Pro Arg Phe Thr Ile Ala Ala Ser Phe Gly
    210                 215                 220

Asn Val His Gly Val Tyr Lys Pro Gly Asn Val Val Leu Thr Pro Thr
225                 230                 235                 240

Ile Leu Arg Asp Ser Gln Glu Tyr Val Ser Lys Lys His Asn Leu Pro
                245                 250                 255

His Asn Ser Leu Asn Phe Val Phe His Gly Gly Ser Gly Ser Thr Ala
            260                 265                 270

Gln Glu Ile Lys Asp Ser Val Ser Tyr Gly Val Val Lys Met Asn Ile
        275                 280                 285

Asp Thr Asp Thr Gln Trp Ala Thr Trp Glu Gly Val Leu Asn Tyr Tyr
    290                 295                 300

Lys Ala Asn Glu Ala Tyr Leu Gln Gly Gln Leu Gly Asn Pro Lys Gly
305                 310                 315                 320

Glu Asp Gln Pro Asn Lys Lys Tyr Tyr Asp Pro Arg Val Trp Leu Arg
                325                 330                 335

Ala Gly Gln Thr Ser Met Ile Ala Arg Leu Glu Lys Ala Phe Gln Glu
            340                 345                 350

Leu Asn Ala Ile Asp Val Leu
        355
```

<210> 11
<211> 921
<212> DNA
<213> Salmonella typhi

<220>
<221> CDS
<222> (1)..(918)

<400> 11

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atg gat atc gcg gtt att ggc tct aac atg gtg gac ctt atc acc tac   48
Met Asp Ile Ala Val Ile Gly Ser Asn Met Val Asp Leu Ile Thr Tyr
  1               5                  10                  15
```

```
acc aac cag atg ccc aaa gaa ggg gaa act ctg gaa gcg ccg gcg ttt   96
Thr Asn Gln Met Pro Lys Glu Gly Glu Thr Leu Glu Ala Pro Ala Phe
             20                  25                  30

aaa atc ggc tgc ggc gga aaa ggg gcg aac cag gcc gtg gcg gcc gct  144
Lys Ile Gly Cys Gly Gly Lys Gly Ala Asn Gln Ala Val Ala Ala Ala
         35                  40                  45

aag ctc aat tca aaa gta ttg atg ttg acc aaa gtg ggc gac gat att  192
Lys Leu Asn Ser Lys Val Leu Met Leu Thr Lys Val Gly Asp Asp Ile
     50                  55                  60

ttt gcc gac aac acc att cgt aat ctc gaa tcc tgg ggg atc aat acg  240
Phe Ala Asp Asn Thr Ile Arg Asn Leu Glu Ser Trp Gly Ile Asn Thr
 65                  70                  75                  80

acg tat gta gaa aaa gta ccg tgt acc agc agc ggc gta gcg ccg att  288
Thr Tyr Val Glu Lys Val Pro Cys Thr Ser Ser Gly Val Ala Pro Ile
                 85                  90                  95

ttc gtc aac gcc aac tcc agc aac agc att ctg atc atc aaa ggc gct  336
Phe Val Asn Ala Asn Ser Ser Asn Ser Ile Leu Ile Ile Lys Gly Ala
            100                 105                 110

aac aag ttt ctc tcg ccg gaa gat atc gat cgc gcg gcg gaa gat tta  384
Asn Lys Phe Leu Ser Pro Glu Asp Ile Asp Arg Ala Ala Glu Asp Leu
        115                 120                 125

aaa aaa tgc cag ctt att gtt ctg caa ctg gaa gtt cag ctt gaa acg  432
Lys Lys Cys Gln Leu Ile Val Leu Gln Leu Glu Val Gln Leu Glu Thr
    130                 135                 140

gtt tat cac gca ata gaa ttt ggc aag aaa cac ggg att gaa gtg tta  480
Val Tyr His Ala Ile Glu Phe Gly Lys Lys His Gly Ile Glu Val Leu
145                 150                 155                 160

tta aac cct gcg cca gca tta cgg gaa tta gat atg tct tat gcc tgt  528
Leu Asn Pro Ala Pro Ala Leu Arg Glu Leu Asp Met Ser Tyr Ala Cys
                165                 170                 175

aaa tgc gat ttc ttt gta cct aat gaa acc gag ctg gaa ata tta acc  576
Lys Cys Asp Phe Phe Val Pro Asn Glu Thr Glu Leu Glu Ile Leu Thr
            180                 185                 190
```

```
ggt atg cca gtg gat acc tat gac cat att cgc gca gcg gca cgt tcg   624
Gly Met Pro Val Asp Thr Tyr Asp His Ile Arg Ala Ala Ala Arg Ser
        195                 200                 205

ctg gta gat aaa ggg ctg aac aat att att gtc acc atg ggc gag aaa   672
Leu Val Asp Lys Gly Leu Asn Asn Ile Ile Val Thr Met Gly Glu Lys
    210                 215                 220

ggc gcg ctg tgg atg acg cgt gac cag gaa gtc cat gtt ccg gcg ttt   720
Gly Ala Leu Trp Met Thr Arg Asp Gln Glu Val His Val Pro Ala Phe
225                 230                 235                 240

aga gtg aac gct gtt gat acc agc ggc gcg ggc gat gcc ttt atc ggc   768
Arg Val Asn Ala Val Asp Thr Ser Gly Ala Gly Asp Ala Phe Ile Gly
                245                 250                 255

tgt ttc gcg cat tac tac gtc cag agc ggg gat gtg gaa gcc gcc atg   816
Cys Phe Ala His Tyr Tyr Val Gln Ser Gly Asp Val Glu Ala Ala Met
            260                 265                 270

aaa aaa gcc gtc ctc ttt gcc gct ttc agc gtc acc ggg aaa ggc acc   864
Lys Lys Ala Val Leu Phe Ala Ala Phe Ser Val Thr Gly Lys Gly Thr
        275                 280                 285

caa tcc tct tat cca agc att gag caa ttt aat gag tat ctt tcg ttg   912
Gln Ser Ser Tyr Pro Ser Ile Glu Gln Phe Asn Glu Tyr Leu Ser Leu
    290                 295                 300

aac gaa taa                                                      921
Asn Glu
305
```

<210> 12
<211> 306
<212> PRT
<213> Salmonella typhi

<400> 12

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Met Asp Ile Ala Val Ile Gly Ser Asn Met Val Asp Leu Ile Thr Tyr
  1               5                  10                  15

Thr Asn Gln Met Pro Lys Glu Gly Glu Thr Leu Glu Ala Pro Ala Phe
            20                  25                  30
```

```
Lys Ile Gly Cys Gly Gly Lys Gly Ala Asn Gln Ala Val Ala Ala Ala
         35                  40                  45

Lys Leu Asn Ser Lys Val Leu Met Leu Thr Lys Val Gly Asp Asp Ile
     50                  55                  60

Phe Ala Asp Asn Thr Ile Arg Asn Leu Glu Ser Trp Gly Ile Asn Thr
 65                  70                  75                  80

Thr Tyr Val Glu Lys Val Pro Cys Thr Ser Ser Gly Val Ala Pro Ile
                 85                  90                  95

Phe Val Asn Ala Asn Ser Ser Asn Ser Ile Leu Ile Ile Lys Gly Ala
            100                 105                 110

Asn Lys Phe Leu Ser Pro Glu Asp Ile Asp Arg Ala Ala Glu Asp Leu
        115                 120                 125

Lys Lys Cys Gln Leu Ile Val Leu Gln Leu Glu Val Gln Leu Glu Thr
    130                 135                 140

Val Tyr His Ala Ile Glu Phe Gly Lys Lys His Gly Ile Glu Val Leu
145                 150                 155                 160

Leu Asn Pro Ala Pro Ala Leu Arg Glu Leu Asp Met Ser Tyr Ala Cys
                165                 170                 175

Lys Cys Asp Phe Phe Val Pro Asn Glu Thr Glu Leu Glu Ile Leu Thr
            180                 185                 190

Gly Met Pro Val Asp Thr Tyr Asp His Ile Arg Ala Ala Ala Arg Ser
        195                 200                 205

Leu Val Asp Lys Gly Leu Asn Asn Ile Ile Val Thr Met Gly Glu Lys
    210                 215                 220

Gly Ala Leu Trp Met Thr Arg Asp Gln Glu Val His Val Pro Ala Phe
225                 230                 235                 240

Arg Val Asn Ala Val Asp Thr Ser Gly Ala Gly Asp Ala Phe Ile Gly
                245                 250                 255

Cys Phe Ala His Tyr Tyr Val Gln Ser Gly Asp Val Glu Ala Ala Met
            260                 265                 270
```

```
Lys Lys Ala Val Leu Phe Ala Ala Phe Ser Val Thr Gly Lys Gly Thr
        275                 280                 285

Gln Ser Ser Tyr Pro Ser Ile Glu Gln Phe Asn Glu Tyr Leu Ser Leu
    290                 295                 300

Asn Glu
305
```

<210> 13
<211> 483
<212> DNA
<213> Lactobacillus leichmannii

<220>
<221> CDS
<222> (10)..(480)

<400> 13

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gtatactaa atg cca aaa aag acg atc tac ttc ggt gcc ggc tgg ttc act 51
          Met Pro Lys Lys Thr Ile Tyr Phe Gly Ala Gly Trp Phe Thr
            1               5                  10

gac cgc caa aac aaa gcc tac aag gaa gcc atg gaa gcc ctc aag gaa   99
Asp Arg Gln Asn Lys Ala Tyr Lys Glu Ala Met Glu Ala Leu Lys Glu
 15                  20                  25                  30

aac cca acg att gac ctg gaa aac agc tac gtt ccc ctg gac aac cag  147
Asn Pro Thr Ile Asp Leu Glu Asn Ser Tyr Val Pro Leu Asp Asn Gln
                35                  40                  45

tac aag ggt atc cgg gtt gat gaa cac ccg gaa tac ctg cat gac aag  195
Tyr Lys Gly Ile Arg Val Asp Glu His Pro Glu Tyr Leu His Asp Lys
            50                  55                  60

gtt tgg gct acg gcc acc tac aac aac gac ttg aac ggg atc aag acc  243
Val Trp Ala Thr Ala Thr Tyr Asn Asn Asp Leu Asn Gly Ile Lys Thr
        65                  70                  75

aac gac atc atg ctg ggt gtc tac atc cct gac gaa gaa gac gtc ggc  291
Asn Asp Ile Met Leu Gly Val Tyr Ile Pro Asp Glu Glu Asp Val Gly
    80                  85                  90
```

```
ctg ggc atg gaa ctg ggt tac gcc ttg agc caa ggc aag tac gtc ctt   339
Leu Gly Met Glu Leu Gly Tyr Ala Leu Ser Gln Gly Lys Tyr Val Leu
 95                 100                 105                 110

ttg gtc atc ccg gac gaa gac tac ggc aag ccg atc aac ctc atg agc   387
Leu Val Ile Pro Asp Glu Asp Tyr Gly Lys Pro Ile Asn Leu Met Ser
                115                 120                 125

tgg ggc gtc agc gac aac gtg atc aag atg agc cag ctg aag gac ttc   435
Trp Gly Val Ser Asp Asn Val Ile Lys Met Ser Gln Leu Lys Asp Phe
            130                 135                 140

aac ttc aac aag ccg cgc ttc gac ttc tac gaa ggt gcc gta tac taa   483
Asn Phe Asn Lys Pro Arg Phe Asp Phe Tyr Glu Gly Ala Val Tyr
        145                 150                 155
```

<210> 14
<211> 157
<212> PRT
<213> Lactobacillus leichmannii

<400> 14

```
Met Pro Lys Lys Thr Ile Tyr Phe Gly Ala Gly Trp Phe Thr Asp Arg
  1               5                  10                  15

Gln Asn Lys Ala Tyr Lys Glu Ala Met Glu Ala Leu Lys Glu Asn Pro
             20                  25                  30

Thr Ile Asp Leu Glu Asn Ser Tyr Val Pro Leu Asp Asn Gln Tyr Lys
         35                  40                  45

Gly Ile Arg Val Asp Glu His Pro Glu Tyr Leu His Asp Lys Val Trp
     50                  55                  60

Ala Thr Ala Thr Tyr Asn Asn Asp Leu Asn Gly Ile Lys Thr Asn Asp
 65                  70                  75                  80

Ile Met Leu Gly Val Tyr Ile Pro Asp Glu Glu Asp Val Gly Leu Gly
                 85                  90                  95

Met Glu Leu Gly Tyr Ala Leu Ser Gln Gly Lys Tyr Val Leu Leu Val
            100                 105                 110
```

Ile Pro Asp Glu Asp Tyr Gly Lys Pro Ile Asn Leu Met Ser Trp Gly
115 120 125

Val Ser Asp Asn Val Ile Lys Met Ser Gln Leu Lys Asp Phe Asn Phe
130 135 140

Asn Lys Pro Arg Phe Asp Phe Tyr Glu Gly Ala Val Tyr
145 150 155

<210> 15
<211> 720
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(717)

<400> 15
atg gct acc cca cac att aat gca gaa atg ggc gat ttc gct gac gta 48
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
1 5 10 15

gtt ttg atg cca ggc gac ccg ctg cgt gcg aag tat att gct gaa act 96
Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
20 25 30

ttc ctt gaa gat gcc cgt gaa gtg aac aac gtt cgc ggt atg ctg ggc 144
Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
35 40 45

ttc acc ggt act tac aaa ggc cgc aaa att tcc gta atg ggt cac ggt 192
Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
50 55 60

atg ggt atc ccg tcc tgc tcc atc tac acc aaa gaa ctg atc acc gat 240
Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
65 70 75 80

ttc ggc gtg aag aaa att atc cgc gtg ggt tcc tgt ggc gca gtt ctg 288
Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
85 90 95

```
ccg cac gta aaa ctg cgc gac gtc gtt atc ggt atg ggt acc tgc acc   336
Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Thr Cys Thr
            100                 105                 110

gat tcc aaa gtt aac cgc atc cgt ttt aaa gac cat gac ttt gcc gct   384
Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125

atc gct gac ttc gac atg gtg cgt aac gca gta gat gca gct aaa gca   432
Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

ctg ggt att gat gct cgc gtg ggt aac ctg ttc tcc gct gac ctg ttc   480
Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

tac tct ccg gac ggc gaa atg ttc gac gtg atg gaa aaa tac ggc att   528
Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175

ctc ggc gtg gaa atg gaa gcg gct ggt atc tac ggc gtc gct gca gaa   576
Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

ttt ggc gcg aaa gcc ctg acc atc tgc acc gta tct gac cac atc cgc   624
Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

act cac gag cag acc act gcc gct gag cgt cag act acc ttc aac aac   672
Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asn
    210                 215                 220

atg atc aaa atc gca ctg gaa tcc gtt ctg ctg ggc gat aaa gag taa   720
Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 16
<211> 239
<212> PRT
<213> Escherichia coli

<400> 16

```
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
  1               5                  10                  15
```

```
Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
            20                  25                  30

Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
        35                  40                  45

Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
    50                  55                  60

Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
65                  70                  75                  80

Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
                85                  90                  95

Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Thr Cys Thr
            100                 105                 110

Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125

Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175

Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asn
    210                 215                 220

Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 17

<211> 1224
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(1221)

<400> 17

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atg aaa cgt gca ttt att atg gtg ctg gac tca ttc ggc atc ggc gct   48
Met Lys Arg Ala Phe Ile Met Val Leu Asp Ser Phe Gly Ile Gly Ala
  1               5                  10                  15

aca gaa gat gca gaa cgc ttt ggt gac gtc ggg gct gac acc ctg ggt   96
Thr Glu Asp Ala Glu Arg Phe Gly Asp Val Gly Ala Asp Thr Leu Gly
             20                  25                  30

cat atc gca gaa gct tgt gcc aaa ggc gaa gct gat aac ggt cgt aaa   144
His Ile Ala Glu Ala Cys Ala Lys Gly Glu Ala Asp Asn Gly Arg Lys
         35                  40                  45

ggc ccg ctc aat ctg cca aat ctg acc cgt ctg ggg ctg gcg aaa gca   192
Gly Pro Leu Asn Leu Pro Asn Leu Thr Arg Leu Gly Leu Ala Lys Ala
     50                  55                  60

cac gaa ggt tct acc ggt ttc att ccg gcg gga atg gac ggc aac gct   240
His Glu Gly Ser Thr Gly Phe Ile Pro Ala Gly Met Asp Gly Asn Ala
 65                  70                  75                  80

gaa gtt atc ggc gcg tac gca tgg gcg cac gaa atg tca tcc ggt aaa   288
Glu Val Ile Gly Ala Tyr Ala Trp Ala His Glu Met Ser Ser Gly Lys
                 85                  90                  95

gat acc ccg tct ggt cac tgg gaa att gcc ggc gtc ccg gtt ctg ttt   336
Asp Thr Pro Ser Gly His Trp Glu Ile Ala Gly Val Pro Val Leu Phe
            100                 105                 110

gag tgg gga tat ttc tcc gat cac gaa aac agc ttc ccg caa gag ctg   384
Glu Trp Gly Tyr Phe Ser Asp His Glu Asn Ser Phe Pro Gln Glu Leu
        115                 120                 125

ctg gat aaa ctg gtc gaa cgc gct aat ctg ccg ggt tac ctc ggt aac   432
Leu Asp Lys Leu Val Glu Arg Ala Asn Leu Pro Gly Tyr Leu Gly Asn
    130                 135                 140
```

```
tgc cac tct tcc ggt acg gtc att ctg gat caa ctg ggc gaa gag cac   480
Cys His Ser Ser Gly Thr Val Ile Leu Asp Gln Leu Gly Glu Glu His
145                 150                 155                 160

atg aaa acc ggc aag ccg att ttc tat acc tcc gct gac tcc gtg ttc   528
Met Lys Thr Gly Lys Pro Ile Phe Tyr Thr Ser Ala Asp Ser Val Phe
                165                 170                 175

cag att gcc tgc cat gaa gaa act ttc ggt ctg gat aaa ctc tac gaa   576
Gln Ile Ala Cys His Glu Glu Thr Phe Gly Leu Asp Lys Leu Tyr Glu
            180                 185                 190

ctg tgc gaa atc gcc cgt gaa gag ctg acc aac ggc ggc tac aat atc   624
Leu Cys Glu Ile Ala Arg Glu Glu Leu Thr Asn Gly Gly Tyr Asn Ile
        195                 200                 205

ggt cgt gtt atc gct cgt ccg ttt atc ggc gac aaa gcc ggt aac ttc   672
Gly Arg Val Ile Ala Arg Pro Phe Ile Gly Asp Lys Ala Gly Asn Phe
    210                 215                 220

caa cgt acc ggt aac cgt cac gac ctg gct gtt gag ccg cca gca ccg   720
Gln Arg Thr Gly Asn Arg His Asp Leu Ala Val Glu Pro Pro Ala Pro
225                 230                 235                 240

acc gtg ctg cag aaa ctg gtt gat gaa aaa cac ggc cag gtg gtt tct   768
Thr Val Leu Gln Lys Leu Val Asp Glu Lys His Gly Gln Val Val Ser
                245                 250                 255

gtc ggt aaa att gcg gac atc tac gcc aac tgc ggt atc acc aaa aaa   816
Val Gly Lys Ile Ala Asp Ile Tyr Ala Asn Cys Gly Ile Thr Lys Lys
            260                 265                 270

gtg aaa gcg act ggc ctg gac gcg ctg ttt gac acc acc atc aaa gag   864
Val Lys Ala Thr Gly Leu Asp Ala Leu Phe Asp Thr Thr Ile Lys Glu
        275                 280                 285

atg aaa gaa gcg ggt gat aac acc atc gtc ttc acc aac ttc gtt gac   912
Met Lys Glu Ala Gly Asp Asn Thr Ile Val Phe Thr Asn Phe Val Asp
    290                 295                 300

ttc gac tct tcc tgg ggc cac cgt cgc gac gtc gcc ggt tat gcc gcg   960
Phe Asp Ser Ser Trp Gly His Arg Arg Asp Val Ala Gly Tyr Ala Ala
305                 310                 315                 320
```

```
ggt ctg gaa ctg ttc gac cgc cgt ctg ccg gag ctg atg tct ctg ctg   1008
Gly Leu Glu Leu Phe Asp Arg Arg Leu Pro Glu Leu Met Ser Leu Leu
                325                 330                 335

cgc gat gac gac atc ctg atc ctc acc gct gac cac ggt tgc gat ccg   1056
Arg Asp Asp Asp Ile Leu Ile Leu Thr Ala Asp His Gly Cys Asp Pro
            340                 345                 350

acc tgg acc ggt act gac cac acg cgt gaa cac att ccg gta ctg gta   1104
Thr Trp Thr Gly Thr Asp His Thr Arg Glu His Ile Pro Val Leu Val
        355                 360                 365

tat ggc ccg aaa gta aaa ccg ggc tca ctg ggt cat cgt gaa acc ttc   1152
Tyr Gly Pro Lys Val Lys Pro Gly Ser Leu Gly His Arg Glu Thr Phe
    370                 375                 380

gcg gat atc ggc cag act ctg gca aaa tat ttt ggt act tct gat atg   1200
Ala Asp Ile Gly Gln Thr Leu Ala Lys Tyr Phe Gly Thr Ser Asp Met
385                 390                 395                 400

gaa tat ggc aaa gcc atg ttc tga                                  1224
Glu Tyr Gly Lys Ala Met Phe
                405
```

<210> 18
<211> 407
<212> PRT
<213> Escherichia coli

<400> 18

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Met Lys Arg Ala Phe Ile Met Val Leu Asp Ser Phe Gly Ile Gly Ala
  1               5                  10                  15

Thr Glu Asp Ala Glu Arg Phe Gly Asp Val Gly Ala Asp Thr Leu Gly
             20                  25                  30

His Ile Ala Glu Ala Cys Ala Lys Gly Glu Ala Asp Asn Gly Arg Lys
         35                  40                  45

Gly Pro Leu Asn Leu Pro Asn Leu Thr Arg Leu Gly Leu Ala Lys Ala
     50                  55                  60

His Glu Gly Ser Thr Gly Phe Ile Pro Ala Gly Met Asp Gly Asn Ala
 65                  70                  75                  80
```

```
Glu Val Ile Gly Ala Tyr Ala Trp Ala His Glu Met Ser Ser Gly Lys
                  85                  90                  95

Asp Thr Pro Ser Gly His Trp Glu Ile Ala Gly Val Pro Val Leu Phe
             100                 105                 110

Glu Trp Gly Tyr Phe Ser Asp His Glu Asn Ser Phe Pro Gln Glu Leu
         115                 120                 125

Leu Asp Lys Leu Val Glu Arg Ala Asn Leu Pro Gly Tyr Leu Gly Asn
     130                 135                 140

Cys His Ser Ser Gly Thr Val Ile Leu Asp Gln Leu Gly Glu Glu His
145                 150                 155                 160

Met Lys Thr Gly Lys Pro Ile Phe Tyr Thr Ser Ala Asp Ser Val Phe
                 165                 170                 175

Gln Ile Ala Cys His Glu Glu Thr Phe Gly Leu Asp Lys Leu Tyr Glu
             180                 185                 190

Leu Cys Glu Ile Ala Arg Glu Glu Leu Thr Asn Gly Gly Tyr Asn Ile
         195                 200                 205

Gly Arg Val Ile Ala Arg Pro Phe Ile Gly Asp Lys Ala Gly Asn Phe
     210                 215                 220

Gln Arg Thr Gly Asn Arg His Asp Leu Ala Val Glu Pro Pro Ala Pro
225                 230                 235                 240

Thr Val Leu Gln Lys Leu Val Asp Glu Lys His Gly Gln Val Val Ser
                 245                 250                 255

Val Gly Lys Ile Ala Asp Ile Tyr Ala Asn Cys Gly Ile Thr Lys Lys
             260                 265                 270

Val Lys Ala Thr Gly Leu Asp Ala Leu Phe Asp Thr Thr Ile Lys Glu
         275                 280                 285

Met Lys Glu Ala Gly Asp Asn Thr Ile Val Phe Thr Asn Phe Val Asp
     290                 295                 300

Phe Asp Ser Ser Trp Gly His Arg Arg Asp Val Ala Gly Tyr Ala Ala
305                 310                 315                 320
```

```
Gly Leu Glu Leu Phe Asp Arg Arg Leu Pro Glu Leu Met Ser Leu Leu
                325                 330                 335

Arg Asp Asp Asp Ile Leu Ile Leu Thr Ala Asp His Gly Cys Asp Pro
            340                 345                 350

Thr Trp Thr Gly Thr Asp His Thr Arg Glu His Ile Pro Val Leu Val
        355                 360                 365

Tyr Gly Pro Lys Val Lys Pro Gly Ser Leu Gly His Arg Glu Thr Phe
    370                 375                 380

Ala Asp Ile Gly Gln Thr Leu Ala Lys Tyr Phe Gly Thr Ser Asp Met
385                 390                 395                 400

Glu Tyr Gly Lys Ala Met Phe
                405
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<210> 19
<211> 780
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(777)

<400> 19

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atg act gat ctg aaa gca agc agc ctg cgt gca ctg aaa ttg atg gac   48
Met Thr Asp Leu Lys Ala Ser Ser Leu Arg Ala Leu Lys Leu Met Asp
  1               5                  10                  15

ctg aac acc ctg aat gac gac gac acc gac gag aaa gtg atc gcc ctg   96
Leu Asn Thr Leu Asn Asp Asp Asp Thr Asp Glu Lys Val Ile Ala Leu
            20                  25                  30

tgt cat cag gcc aaa act ccg gtc ggc aat acc gcc gct atc tgt atc   144
Cys His Gln Ala Lys Thr Pro Val Gly Asn Thr Ala Ala Ile Cys Ile
        35                  40                  45

tat cct cgc ttt atc ccg att gct cgc aaa act ctg aaa gag cag ggc   192
Tyr Pro Arg Phe Ile Pro Ile Ala Arg Lys Thr Leu Lys Glu Gln Gly
    50                  55                  60
```

```
acc ccg gaa atc cgt atc gct acg gta acc aac ttc cca cac ggt aac   240
Thr Pro Glu Ile Arg Ile Ala Thr Val Thr Asn Phe Pro His Gly Asn
 65                  70                  75                  80

gac gac atc gac atc gcg ctg gca gaa acc cgt gcg gca atc gcc tac   288
Asp Asp Ile Asp Ile Ala Leu Ala Glu Thr Arg Ala Ala Ile Ala Tyr
                85                  90                  95

ggt gct gat gaa gtt gac gtt gtg ttc ccg tac cgc gcg ctg atg gcg   336
Gly Ala Asp Glu Val Asp Val Val Phe Pro Tyr Arg Ala Leu Met Ala
            100                 105                 110

ggt aac gag cag gtt ggt ttt gac ctg gtg aaa gcc tgt aaa gag gct   384
Gly Asn Glu Gln Val Gly Phe Asp Leu Val Lys Ala Cys Lys Glu Ala
        115                 120                 125

tgc gcg gca gcg aat gta ctg ctg aaa gtg atc atc gaa acc ggc gaa   432
Cys Ala Ala Ala Asn Val Leu Leu Lys Val Ile Ile Glu Thr Gly Glu
    130                 135                 140

ctg aaa gac gaa gcg ctg atc cgt aaa gcg tct gaa atc tcc atc aaa   480
Leu Lys Asp Glu Ala Leu Ile Arg Lys Ala Ser Glu Ile Ser Ile Lys
145                 150                 155                 160

gcg ggt gtg gac ttc atc aaa acc tct acc ggt aaa gtg gct gtg aac   528
Ala Gly Val Asp Phe Ile Lys Thr Ser Thr Gly Lys Val Ala Val Asn
                165                 170                 175

gcg acg ccg gaa agc gcg cgc atc atg atg gaa gtg atc cgt gat atg   576
Ala Thr Pro Glu Ser Ala Arg Ile Met Met Glu Val Ile Arg Asp Met
            180                 185                 190

ggc gta gaa aaa acc gtt ggt ttc aaa ccg gcg ggc ggc gtg cgt act   624
Gly Val Glu Lys Thr Val Gly Phe Lys Pro Ala Gly Gly Val Arg Thr
        195                 200                 205

gcg gaa gat gcg cag aaa tat ctc gcc att gca gat gaa ctg ttc ggt   672
Ala Glu Asp Ala Gln Lys Tyr Leu Ala Ile Ala Asp Glu Leu Phe Gly
    210                 215                 220

gct gac tgg gca gat gcg cgt cac tac cgc ttt ggc gct tcc agc ctg   720
Ala Asp Trp Ala Asp Ala Arg His Tyr Arg Phe Gly Ala Ser Ser Leu
225                 230                 235                 240
```

```
ctg gca agc ctg ctg aaa gcg ctg ggt cac ggc gac ggt aag agc gcc   768
Leu Ala Ser Leu Leu Lys Ala Leu Gly His Gly Asp Gly Lys Ser Ala
                245                 250                 255

agc agc tac taa                                               780
Ser Ser Tyr
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<210> 20
<211> 259
<212> PRT
<213> Escherichia coli

<400> 20

```
Met Thr Asp Leu Lys Ala Ser Ser Leu Arg Ala Leu Lys Leu Met Asp
  1               5                  10                  15

Leu Asn Thr Leu Asn Asp Asp Asp Thr Asp Glu Lys Val Ile Ala Leu
             20                  25                  30

Cys His Gln Ala Lys Thr Pro Val Gly Asn Thr Ala Ala Ile Cys Ile
         35                  40                  45

Tyr Pro Arg Phe Ile Pro Ile Ala Arg Lys Thr Leu Lys Glu Gln Gly
     50                  55                  60

Thr Pro Glu Ile Arg Ile Ala Thr Val Thr Asn Phe Pro His Gly Asn
 65                  70                  75                  80

Asp Asp Ile Asp Ile Ala Leu Ala Glu Thr Arg Ala Ala Ile Ala Tyr
                 85                  90                  95

Gly Ala Asp Glu Val Asp Val Val Phe Pro Tyr Arg Ala Leu Met Ala
            100                 105                 110

Gly Asn Glu Gln Val Gly Phe Asp Leu Val Lys Ala Cys Lys Glu Ala
        115                 120                 125

Cys Ala Ala Ala Asn Val Leu Leu Lys Val Ile Ile Glu Thr Gly Glu
    130                 135                 140

Leu Lys Asp Glu Ala Leu Ile Arg Lys Ala Ser Glu Ile Ser Ile Lys
145                 150                 155                 160
```

```
Ala Gly Val Asp Phe Ile Lys Thr Ser Thr Gly Lys Val Ala Val Asn
                165                 170                 175

Ala Thr Pro Glu Ser Ala Arg Ile Met Met Glu Val Ile Arg Asp Met
            180                 185                 190

Gly Val Glu Lys Thr Val Gly Phe Lys Pro Ala Gly Gly Val Arg Thr
        195                 200                 205

Ala Glu Asp Ala Gln Lys Tyr Leu Ala Ile Ala Asp Glu Leu Phe Gly
    210                 215                 220

Ala Asp Trp Ala Asp Ala Arg His Tyr Arg Phe Gly Ala Ser Ser Leu
225                 230                 235                 240

Leu Ala Ser Leu Leu Lys Ala Leu Gly His Gly Asp Gly Lys Ser Ala
                245                 250                 255

Ser Ser Tyr
```